Vol. LI *August, 1926* *No. 2*

BIOLOGICAL BULLETIN

OBSERVATIONS ON MOTILITY IN CERTAIN CYANOPHYCEÆ.[1]

EDWARD S. CASTLE.

The power of spontaneous movement in the blue-green algæ has been stated by many authors to be confined to the family Oscillatoriaceæ, occurring characteristically in the vegetative filaments of *Oscillatoria*, *Phormidium*, *Spirulina* and *Arthrospira*, and occasionally in the hormogonia of related genera and even of such Heterocysteæ as *Nostoc*, *Scytonema*, and *Rivularia*. In addition, DeBary (1863) and Phillips (1904) have described motion in vegetative filaments of the genus *Cylindrospermum*, a member of the Nostocaceæ.

Recently, however, two species of *Anabæna* occurring on the estate of Mr. E. F. Atkins at Soledad, Cuba, and studied by the writer in the Harvard Laboratory there, were found, although belonging to the Nostocaceæ, to show consistent motility like that characteristic of the Oscillatoriaceæ. These two species were both commonly collected, forming thin scums on damp earth in roadside ditches. In culture bottles they spread rapidly up the sides of the glass in a manner resembling an active *Oscillatoria*, finally covering the whole inside surface with filaments packed side by side in a single layer. Although exhibiting an active motility like that regarded as characteristic of members of the Oscillatoriaceæ, the filaments of both these species were torulose and consistently contained heterocysts that conclusively excluded them from that family. The larger of the two forms (Fig. 1*b*) regularly produced spores and in all essential characteristics justified being identified as a species of *Anabæna;* the other, smaller form (Fig. 1*a*), although it never developed spores during

[1] Studies from the Biological Laboratory in Cuba (Atkins Foundation) of the Harvard Institute for Tropical Biology and Medicine, No. 1.

the two months it was under observation, nevertheless was judged an *Anabæna* on its general morphology. Exact determination of the species was not attempted by the writer but will be referred later to specialists in the classification of the group.

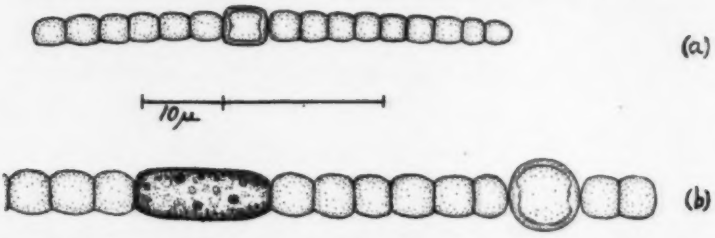


FIG. 1. Portions of the trichomes of the motile species of *Anabæna* studied at Soledad. (*a*) The smaller species showing its characteristic structure. (*b*) The larger species showing the cells, heterocyst, and spore in their characteristic arrangement.

Both these species of *Anabæna* were consistently and actively motile, the motility resembling in general that customary in the Oscillatoriaceæ. Flexion of the whole filament as well as translatory movement was seen in both species, but rotation was not observed. Filaments with heterocysts moved as actively as those without, contrary to what Borzi (1886) observed in the case of species of *Nostoc*. Speed of translation was, within limits, independent of the length of the filament, confirming the observations of Crozier and Federighi (1924) on *Oscillatoria*. At 28.9° C. (84° F.), the rates of translation of these species of *Anabæna*, compared with the rates of some rapidly-motile species of *Oscillatoria*, were:

Anabæna sp.	av. 2.5 μ per sec.
Oscillatoria formosa	" 4.2 μ " "
Oscillatoria princeps	" 5.0 μ " "

The rate of movement in *Anabæna* is thus seen to be half that of *Oscillatoria princeps* and somewhat more than half that of *Oscillatoria formosa* under the same conditions. Although motility has not been reported in *Anabæna* hitherto, the motility which has been recorded frequently in the hormogonia or such related Heterocysteæ as *Nostoc*, *Scytonema*, or *Rivularia* has in-

variably been described as of a very slow nature. The rates given by West (1916, p. 23), as found by Brand for the hormogonia of species of *Phormidium*, vary from 0.004 to 0.058 μ per second, and are thus many times slower than those seen by the writer in the vegetative filaments of *Anabæna;* but it should be noted that the temperature at which these slower rates occurred was not given.

In view of the fact that during the movement of these species of *Anabæna* no rotation was ever observed by the writer, it seemed advisable for comparison to examine species of *Oscillatoria* in this respect, especially since Crozier and Federighi (*loc. cit.*) have expressed some doubt whether rotation of the filaments of *Oscillatoria* does indeed always take place during translation. Careful examination of a considerable number of favorable species of *Oscillatoria* at Soledad has convinced the writer most conclusively that many species do exhibit rotation of the filament around the long axis. In species in which the ends of the filaments were characteristically hooked, this hook, which maintained its peculiar shape, could be seen to rotate through the water in a manner precluding a mere circular bending of the tip. Moreover, in larger species, rotation could be observed very convincingly by following the change in relative position of granules lying at different depths within the cell content.

Also, in view of the fact that West (1916 and 1904), when discussing the motility of *Arthrospira*, states that rotation has not been observed in this genus, it may be of interest to record that in a species studied at Soledad, rotation was found to accompany progression, as in *Spirulina*, while flexion also was seen to occur at times.

Summary.

Two species of *Anabæna*, of the family Nostocaceæ, studied in Cuba, were found to exhibit motion similar to that of the Oscillatoriaceæ. The rate of movement was independent of the presence of heterocysts or of the length of the filament, and was about one half that of actively motile Oscillatoriæ under the same conditions, but much more rapid than that previously recorded for the hormogonia of *Phormidium*. No rotation was observed in these species of *Anabæna* but several species of

Oscillatoria and one species of *Arthrospira* were seen to rotate customarily during free-swimming translation when observed under similar conditions.

REFERENCES.

Borzi.

'86 Le Communicazioni intracellulari delle Nostochinee. Malpighia, 1. (Reviewed in Bot. Cent., 37, 1887.)

Crozier, W. J., and Federighi, H.

'24 Critical Thermal Increment for the Movement of Oscillatoria. Journ. Gen. Physiol., 7, 137–150.

De Bary, A.

'63 Beitrag zur Kentniss der Nostocaceæ insbesonderbare der Rivularien. Flora.

Phillips, O. P.

'04 A Comparative Study of the Cytology and Movements of the Cyanophyceæ. Contrib. Bot. Lab. Univ. of Penna., Vol. II., No. 3.

West, G. S.

'04 British Freshwater Algæ.

'16 Cambridge Botanical Handbooks, Vol. 1, Algæ.

NUMBER AND BEHAVIOR OF THE CHROMOSOMES IN *CAVIA COBAYA*[1] (THE COMMON GUINEA PIG).

MARY T. HARMAN AND FRANK P. ROOT,

KANSAS STATE AGRICULTURAL COLLEGE.[2]

INTRODUCTION 73
MATERIAL AND METHODS 74
DESCRIPTION OF MATERIAL 75
1. Spermatogonial divisions 75
2. The growth period 75
3. Spermatocyte divisions 76
DISCUSSION 77
SUMMARY 79
LITERATURE CITED 79
DESCRIPTION OF PLATES 82

INTRODUCTION.

Since the guinea pig has been used extensively in genetic experiments and since investigation has revealed in many instances a close correlation between the genetic behavior and the number and behavior of the chromosomes of many organisms, the number and behavior of the chromosomes of the guinea pig is of interest alike to the cytologist and the geneticist.

According to Harvey (1920) there is a wide difference of opinion concerning the number of chromosomes observed in *Cavia*. Von Bardeleben (1892) reports sixteen spermatogonial chromosomes and eight chromosomes in the spermatids. Flemming (1898) says that there are probably twenty-four somatic chromosomes. Moore and Walker (1906) give the number as thirty-two in the spermatogonial cells and sixteen in the primary and secondary spermatocyte cells. Stevens (1911) says that there are about fifty-six spermatogonial chromosomes and twenty-eight chromosomes in both the primary and secondary spermato-

[1] Dr. H. L. Ibsen furnished the guinea pigs for this study. P. W. Gregory assisted in the fixation and dehydration. The drawings were made by S. Fred Prince.

[2] Contribution from the Zoölogical Laboratory, Kansas State Agricultural College No. 851.

cytes. She further states that the sex chromosomes are of the XY-type which are unequal in size and separate in the first spermatocyte division. Athias (1912) reports twenty-four to twenty-eight primary spermatocyte chromosomes in *Cavia porcellus* and perhaps twenty-four secondary spermatocyte chromosomes. Lams (1913) says that there are sixteen somatic chromosomes, and eight primary and secondary spermatocyte chromosomes and eight chromosomes in the spermatids. In most cases the material has been reported as very unfavorable or the count doubtful. Our counts do not agree with any of those reported previously. We may add, however, that in many instances our material is almost diagrammatically clear. We have found thirty-eight spermatogonial chromosomes and nineteen chromosomes in the primary and secondary spermatocyte cells. Like Stevens, we have found an XY-chromosome which separates in the first maturation division.

Material and Methods.

The present paper is limited to the number and behavior of the spermatogonial and spermatocyte chromosomes. We have found some interesting things in connection with the transformation of the spermatid which we hope to give in another paper.

Two male guinea pigs were used for this study. One of these animals was superactive sexually and the other was a normally active male. The testes were removed and cut into small sections. These were placed in cold Flemming's solution to which urea had been added. This was done according to the technique of Hance (1917) with the exception that the temperature was 2° C. instead of 4° C. The fixing fluid had been out of doors several hours previous to using it. The material was fixed for about twenty-four hours. The temperature was almost constant during this time. The pieces of fixed testes were transferred to water at room temperature for twenty-four hours. The water was changed frequently. Dehydration was gradual, although the drop method was not employed. Xylol was used for clearing and paraffin was the embedding medium. The sections were cut from three to ten micra. Those sections from

three to seven micra proved best for study. It was difficult to isolate individual cells in thicker sections. Sections three micra did not seem to be particularly advantageous over those five or six micra. The most favorable stain was Heidenhain's iron-alum hematoxylin.

Description of Material.

It was found that the actively dividing cells are not distributed throughout the whole lining of the seminiferous tubules but are in very restricted areas. These areas are larger and more numerous in the superactive male. They are elliptical in shape. The greatest diameter is always lengthwise of the tubule. This dimension never exceeds two thirds of the circumference of the tubule and may be as small as only a few cells.

1. *Spermatogonial Divisions.*

Many and repeated counts have given us thirty-eight spermatogonial chromosomes. Eight of these are distinctly U-shaped which may be arranged in four pairs. Twenty-eight chromosomes are slightly bent rods of various sizes which, according to size and shape, may be arranged in pairs. The remaining two chromosomes are unequal in size and irregular in shape. We have designated these as the XY-pair. Fig. 2 is a polar view of a metaphase plate of a spermatogonial division. The chromosomes which form the members of a given pair have been designated by the same number. Reference to this figure shows that the chromosomes are not arranged merely around the periphery of the spindle as is so often the case in insects (Harman, 1915 and 1920), but fill in the entire diameter of the spindle. No one kind of chromosome occupies one area of the spindle exclusively. The large, slightly bent rods are deep in the spindle as well as around the periphery. The same may be said of the U-shaped chromosomes. However, the relative arrangement of these various chromosomes is approximately the same.

2. *The Growth Period.*

Evidently a longer period of time is spent in the stages between the last spermatogonial division and the first spermatocyte division than in any other stage, for there are always many times

more cells in these stages than the other stages. This is true, not only for the animals used in this study, but also for other guinea pigs which we have examined.

In the early prophase of the primary spermatocyte cells the chromatin is coarsely granular and is in an indistinct reticulum. There is no uniformity in either the size or the shape of these granules in a particular cell, but there are always a few which are distinctly larger than the majority and decidedly more compact. As the nucleus increases in size, the chromatin granules become smaller and the reticulum less distinct until the nucleus is almost clear, except for here and there masses of chromatin of loose construction. After this the nucleus does not get much larger but the chromatin material becomes more concentrated near one side of the nucleus until it becomes a tangled mass in which it is difficult to distinguish any particular parts or any definite arrangement. Gradually this mass loosens up and more nearly fills the entire nucleus. In addition to the reticulum there is a number of more compact particles of chromatin material. These seem to be the beginnings of the bivalent chromosomes. Long before all the chromatin material has been formed into definite chromosomes each of these masses appears double. Fig. 3 shows nineteen of these double chromosomes with the chromatin in so loose a condition that each body has a woolly appearance. Fig. 5 is a later stage in which the chromatin is more compact and the double condition is no longer apparent. Figs. 4 and 6 are fully formed primary spermatocyte chromosomes. By reference to these figures it will be seen that no two chromosomes are of exactly the same shape or size. As was found in the spermatogonial divisions the spermatocyte chromosomes are distributed through the entire diameter of the spindle.

3. *Spermatocyte Divisions.*

Figure 7 is a lateral view of a metaphase plate of a primary spermatocyte division. It will be noted that one chromosome is almost divided and that the division is an unequal one. We have thought that this is the XY-chromosome. We have designated the larger component as the X and the smaller one as the Y. Fig. 8 shows the autosomes undivided while the XY has com-

pleted its division and each component is half way to the poles. Fig. 10 is an anaphase of a primary spermatocyte division. This figure shows a lack of synchronism in the division of the autosomes.

Figure 9 is an anaphase of a secondary spermatocyte division. It will be seen that some of the chromosomes divide much in advance of the others. Figs. 11 and 12 are polar views of metaphase plates of the secondary spermatocyte divisions. In each of these figures are seen nineteen chromosomes. Fig. 12 is not a direct polar view but is a view of the metaphase plate at a tangent.

Discussion.

The large number of chromosomes, the comparative small size and the tendency of the chromatin material to mass together and form into clumps in which the individual parts are not distinguishable have made the study of mammalian chromosomes a difficult task. This may, in part, account for the differences in the interpretation of the chromosome complex in observations on the same species as was cited in the beginning of this paper. Other difficulties are the scarcity of cells in the various stages of active division, the lack of synchronism in the division of the chromosomes and the distribution of the chromosomes throughout the entire diameter of the spindle.

We are of the opinion that a selection of animals which show sexual activity at the time of securing the material greatly increases the size and number of areas of cells in active stages of division. We found this true not only for the animals used in this study but also for a number of other guinea pigs which we have observed. This also agrees with Painter's (Painter, 1924) observations on the monkey and other mammals.

Since the chromosomes are closely packed together the selection of a fixing agent which does not swell the chromatin material is very desirous. We have found that the modification of Flemming's solution with urea used at a temperature slightly above freezing gives the most satisfactory results. We have used Allen's modification of Bouin (Allen, 1919) at about body temperature with less satisfactory results. This is the technique which Painter has used so successfully.

Most of the cells in the actively dividing stages of our material are diagrammatically clear. Owing to the fact that some of the chromosomes are precocious and others are lagging, many of the polar views of metaphase plates do not contain all the chromosomes.

It is of some interest to note that although there is a great difference in the number of chromosomes reported for the guinea pig, no one has reported an unpaired chromosome and only Stevens (1911) has previously reported one which divides unequally. This is the condition which Painter (1923 and 1924) has found for the opossum, two species of monkeys, the horse and both the negro and the white man. However, it differs from the findings of Wodsedalek (1914) and Masui (1919) for the horse. Since the female has not been studied the basis for calling this pair of unequally dividing chromosomes the XY-pair is the behavior. We have chosen to call the large component the X-element and the smaller component the Y-element because this is the condition in those animals in which the homologues have been studied in the female. It is perfectly possible that an examination of the female might reveal the opposite condition. This is of further interest because it is cytological evidence that the male guinea pig is heterozygous for sex which has been taken for granted among geneticists but which has not been shown experimentally. Known sex-linked characters in the mammals are few. They have been found in man and the cat.

We have found no indication of telosynapsis as was described by Moore and Walker (1906). They state that "In the guinea pig there are, as we have seen, 32 premaiotic chromosomes, and the synaptic loops of the first maiotic division resolved themselves into 16 gemini, so that we are led to conclude that gemini consist each of two somatic chromosomes joined end to end." Neither have we found a condition which could certainly be interpreted as parasynapsis. The condition illustrated in Fig. 3 might be interpreted as parasynapsis, but could as readily be considered as a precocious splitting of the chromosomes in tetrad formation. The fact that there are four U-shaped primary spermatocyte chromosomes and that there are eight similar shaped spermatogonial chromosomes would be evidence in favor

of parasynapsis. There is no genetic evidence in favor of parasynapsis.

Summary.

1. The actively dividing cells are not distributed throughout the whole lining of the seminiferous tubules but are limited to elliptical areas. 2. These areas never exceed two thirds the circumference of the tubules and sometimes consist of only a few cells. 3. The greatest diameter of the ellipse is always lengthwise of the tubule. 4. The areas of actively dividing cells are more numerous and larger in the superactive male. 5. There are thirty-eight spermatogonial chromosomes, which according to size and shape may be grouped into eighteen similar pairs and a pair which is unequal in size which we have designated as the XY-pair. 6. Both the primary and secondary spermatocyte cells have nineteen chromosomes. 7. The XY-tetrad is precocious in its division and the resultant chromosomes are unequal in size. 8. All the chromosomes divide equally in the second division. 9. There is some evidence of parasynapsis.

Bibliography.

Athias, M.

'12 Sur les divisions de maturation de l'œuf des mammiferes. Archivos do Instituto bacteriologieo Camara Pestana, Tome 3.

Bardeleben, K. von.

'92 Ueber Spermatogense bei Säugetieren. Verb. d. Anat. Ges.

Flemming, W. von.

'98 Ueber die Chromosomenzahl beim Menschen. Anat. Anz. Bd. 14.

Hance, Robert T.

'17 The Diploid Complex of the Pig. Jour. Morph., Vol. 30.

'17 The Fixation of Mammalian Chromosomes. Anat. Rec., Vol. 12.

Harman, Mary T.

'15 Spermatogenesis in *Paratettix*. Biol. Bull., Vol. 29.

'20 Chromosome Studies in Tettigidæ. II. Chromosomes in Paratettix BB and CC and their Hybrid BC. Biol. Bull., Vol. 38.

Harvey, Ethel Brown.

'20 A Review of Chromosome Numbers in the Metazoa. Part II. Jour. Morph., Vol. 34.

Lams, Honore.

'18 Etude de l'œuf de Cobaya aux premiers stades de l'embryogenèse. Archives de Biologie, Vol. 28.

Masui.

'19 Jour. Imp. Univ. Agri. Tokyo, Vol. 3.

Moore and Walker.

'06 The Maiotic Process in Mammalia. Thompson Yates and Johnston Laboratories, University of Liverpool, Report 7.

Painter, Theophilus S.

'21 The Y-chromosomes in Mammals. Science, N.S., Vol. 53.

'22 Studies in Mammalian Spermatogenesis. I. The Spermatogenesis of the Opossum. Jour. Exp. Zoöl., Vol. 35.

'23 Studies in Mammalian Spermatogenesis. II. The Spermatogenesis of Man. Jour. Exp. Zoöl., Vol. 37.

'24 Studies in Mammalian Spermatogenesis. IV. The Sex Chromosomes of Monkeys. Jour. Exp. Zoöl., Vol. 39.

'24 Studies in Mammalian Spermatogenesis. V. The Chromosomes of the Horse. Jour. Exp. Zoöl., Vol. 39.

Stevens, N. M.

'11 Heterochromosomes in the Guinea Pig. BIOL. BULL., Vol. 21.

Wodsedalek, J. E.

'14 Spermatogenesis of the Horse. BIOL. BULL., Vol. 27.

EXPLANATION OF PLATES.

All the drawings were made with the aid of a camera lucida, a 1.9 oil immersion objective and a number 10 ocular at table level. Then they were enlarged two diameters. The reproductions were reduced $\frac{1}{3}$ from the original.

PLATE I.

FIG. 1. Spireme of spermatogonial division.

FIG. 2. Metaphase plate polar view of spermatogonial division showing nineteen pairs of chromosomes. The pair numbered 19 is the XY-pair.

FIG. 3. Formation of the primary spermatocyte chromosomes.

FIG. 4. Metaphase plate of primary spermatocyte chromosome showing the unequal XY-tetrad.

FIG. 5. Formation of primary spermatocyte chromosomes. This is a later stage than Fig. 3.

FIG. 6. Metaphase plate of primary spermatocyte chromosomes.

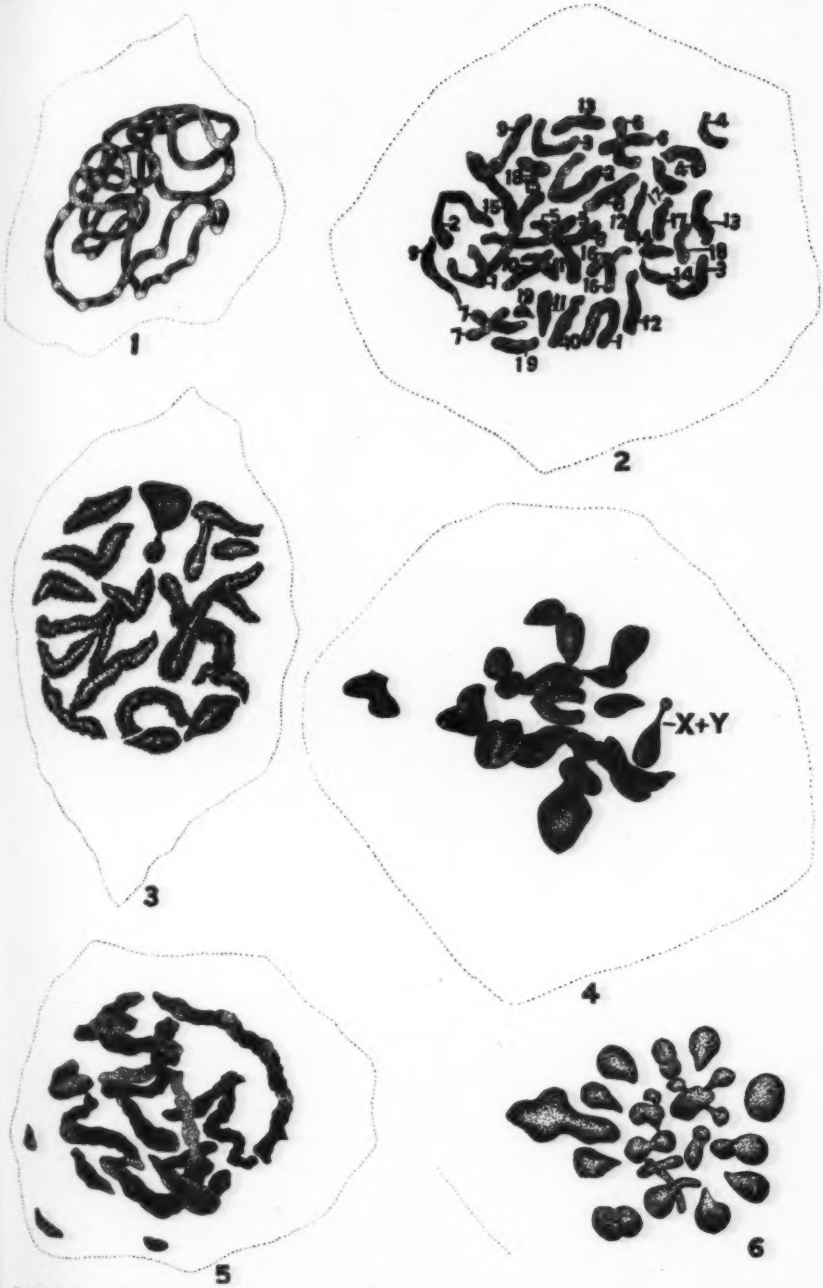

1
2
3
4
-X+Y
5
6

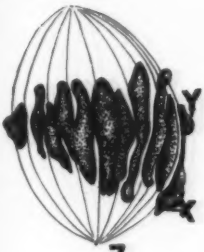
PLATE II.

FIG. 7. Metaphase plate lateral view of primary spermatocyte showing the precocious and unequal division of the XY-tetrad.

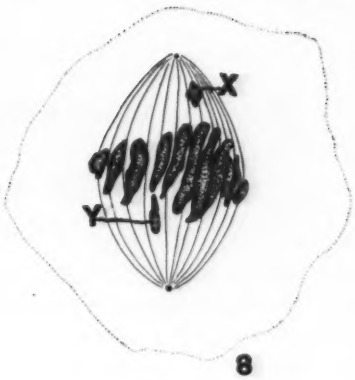
FIG. 8. Same as 7, except the XY has entirely divided.

FIGS. 9 AND 10. Anaphase primary spermatocyte.

FIGS. 11 AND 12. Metaphase plate polar view of secondary spermatocyte divisions.

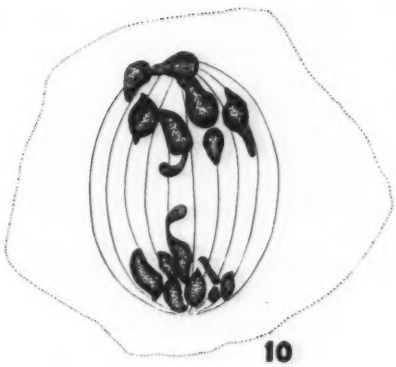


7



8

9



10

11

12

UNIV. OF MICH.

ON THE INHIBITION OF ANIMAL LUMINESCENCE BY LIGHT.

E. NEWTON HARVEY,[1]

PRINCETON UNIVERSITY.

Since Allman's (1862) observations on Ctenophores, many researches have left no doubt of the inhibition of the luminescence of these forms by light. There is not only inhibition of the irritability but an actual disappearance of photogenic substance, which will return again in the dark, even in extracts of Ctenophores, provided the photogenic cells have not been entirely broken up. But a centrifuged extract of Ctenophores exposed to sunlight shows only slight recovery of its power to luminesce in the dark, as compared with a control kept continually in the dark. The centrifuged extract contains photogenic granules but not photogenic cells, and I think it doubtful if isolated granules recover the power to luminesce, although this point is difficult to ascertain with certainty.

Heinemann (1872) observed inhibition of the luminescence of *Pyrophorus*, the West Indian elaterid, and I have observed marked inhibition of the luminous extract of *Cypridina* (Harvey, 1925) but none of the whole living animals themselves.

Massart (1893) found a day and night periodicity of luminescence in *Noctiluca*, Zacharias (1905) in *Ceratium*, B. Moore (1908) in some animals of the plankton, presumably copepods, and Heymans and A. R. Moore (1923) in *Pelagia noctiluca*, a medusa. It is reported that these forms will not luminesce in the daytime even when they have been kept in a dark room. In a recent note A. R. Moore (1926) was unable to confirm the observations of Heymans and Moore in *Pelagia* and I have also been unable to observe any inhibiting effect of strong sunlight on either the local or general luminescence of this form, although the exposure was from sunrise until noon of a cloudless day in December. Parker (1920) finds inhibition of luminescence of the pennatulid, *Renilla*, by sunlight.

[1] Appointed to the A. A. A. S. table at Naples, Oct. 1925–Feb. 1926.

During the past winter at Naples I had an opportunity of examining many luminous forms after exposure to light and these observations, together with previous ones, are collected in this paper. As sources of light the sun (between 10 A.M. and noon), a large carbon arc and a small carbon arc were used. All the light passed through glass and water to absorb the heat. Light from the carbon arcs was condensed by glass lenses. The large arc gave (judging by its inhibition of *Cypridina* luminescence) at least 10,000 foot-candles while the smaller one, perhaps half of that. Either the whole animal or extracts of luminous material were exposed for the times indicated.

As to periodicity of luminescence, I have never yet observed an animal which would not luminesce during the daytime, provided it had been kept in the dark for a few hours previously. I have never studied the dinoflagellates but the copepods at Naples (Dec., Jan.), *Noctiluca miliaris* at Misaki, Japan (July), and Friday Harbor, Washington (Aug.), *Balanoglossus minutus* at Naples (Dec.), *Ptychodera* sp. at Bermuda (Dec.), and *Pelagia noctiluca* at Naples (Dec.) luminesce during the day.

Other forms in which the *whole animal* has been exposed to sunlight for the time indicated, and shows no inhibition of luminescence when tested immediately in a dark room are as follows: the ophiurian, *Amphiura squamata* (Naples, Dec., 15 min.); the annelids, *Chætopterus variopedatus* (Woods Hole, July) and *Microscolex phosphorea* (Naples, Dec., 20 min.); the radiolaria, *Thalassicolla nucleata*, *Colozoun inerme* and *Sphærozoön punctatum* (Naples, Dec. 15 min.); luminous bacteria; luminous fungus (*Panus stipticus*) (Woods Hole, Aug.); pennatulids, *Pennatula phosphorea* (Naples, Dec.), *Cavernularia haberi* (Misaki, Japan, July), *Ptylosarcus* sp. (Friday Harbor, Wash., July); *Pyrosoma* sp. (Monaco, Jan., 60 min.); *Cypridina hilgendorfii* (Misaki, Japan, July); the medusæ, *Mitrocoma cellularia*, *Phialidium gregarium*, *Stomatoca atra*, and *Æquora forskalia* (Friday Harbor, Wash., Aug.); the squid, *Watasenia scintillans* (Japan, May); the fish, *Monocentris japonica;* and the fire-flies, *Luciola parva* and *viticollis* (Japan, July).

Forms in which a luminous extract has been exposed to the carbon arc and in which no inhibition is to be noted are: *Pholas*

dactylus (large arc, 4 min.); *Heteroteuthis dispar* (small arc, 3 min.).

As to inhibition of fireflies, I have observed no inhibition of the luminous organ of *Photuris pennsylvanica* removed from the body and mashed on a slide (so that it luminesced continuously) after 4 minutes' exposure to 15,000 foot-candles. However, when the luminous organ (alone) in the intact animal is directly exposed to the above light, the normal flashes of the animal as well as the flashes from electrical stimulation (of the thorax) are inhibited and remain so for some seconds after the light is screened. Illumination of the eyes by 15,000 foot-candles while keeping the luminous organ in the dark does not inhibit the flashing.

Pyrophorus noctilucus sent to Woods Hole from Cuba shows no inhibition of the teased organ in 15,000 foot-candles illumination but if one of the two thoracic organs is exposed to sunlight or the carbon arc light (passing through water) for two minutes, while it is glowing steadily, it will be observed that this exposed organ now glows less strongly than the other in the dark.

We are evidently dealing in these cases with no true inhibition of luminescence of luminous material but with an effect of light on the local nerve mechanism of stimulation in the organ itself, a process quite different from that in *Cypridina*.

It will thus be observed that inhibition of luminescence is rather rare and that Ctenophores and *Cypridina* remain the best known cases. *Noctiluca* and the Dinoflagellates need re-investigation and it is possible that some species of copepods show inhibition. A large number of other forms do not.

In *Cypridina* the inhibition is due to acceleration of the spontaneous (without luminescence) oxidation of luciferin. Light has no inhibiting effect in absence of oxygen. This probably explains why the whole *Cypridinas* are not inhibited, for the great mass of luciferin must be kept under anaërobic conditions in the gland.

I have also investigated the Ctenophores to determine if their luminescence was inhibited by light in absence of oxygen and was surprised to find inhibition by light when the last traces of oxygen were removed from a *Beroë*, *Eucharis* or *Mnemiopsis*

extract, with platinized asbestos and hydrogen. I later discovered that Ctenophore extracts will luminesce in complete absence of oxygen [1] and presume that oxygen must be bound in some way with the photogenic granules. It is quite possible, then, that the inhibition by light in Ctenophores may also be due to photochemical acceleration of oxidation (without luminescence) of photo-genic material.

Conclusions.

Inhibition of luminescence of photogenic material by light is not a general phenomenon. Ctenophores remain the best known case, but in my experience pennatulids and medusæ show no inhibition. *Cypridina* extracts are also inhibited if they contain oxygen and the inhibition seems to consist of an oxidative destruction of photogenic substance.

I have never yet observed an animal that would fail to luminesce in the daytime provided it had been kept in darkness for a few hours previously. Flagellates and copepods should be studied further to test the question of day-night rhythm of luminescence.

References.

Allman, G. I.
'62 Proc. Roy. Soc. Edinb., IV., 518.
Harvey, E. N.
'25 Journ. Gen. Physiol., VII., 679.
Heinemann, C.
'72 Arch. f. Mikr. Anat., VIII., 461, and XXVII., 296.
Heymans, C., and Moore, A. R.
'23 Journ. Gen. Phys., VI., 273.
Massart, J.
'93 Bull. sc. de France et de la Belgique, XXV.
Moore, A. R.
'26 Journ. Gen. Physiol., IX., 375.
Moore, B.
'08 Bioc. Journ., IV., 1.
Parker, G. H.
'20 Proc. Amer. Phil. Soc., XIX., 171
Zacharias, O.
'05 Biol. Centralb., XXV., 20.

[1] Not yet published.

OXYGEN AND LUMINESCENCE, WITH A DESCRIPTION OF METHODS FOR REMOVING OXYGEN FROM CELLS AND FLUIDS.

E. NEWTON HARVEY,[1]

PRINCETON UNIVERSITY.

(From the Stazione Zoölogica, Naples.)

So many luminous animals require dissolved gaseous oxygen for luminescence that one might expect all to need it, but I have recently discovered certain forms which can luminesce without oxygen. Perhaps they utilize oxygen already bound up with the luminous material or perhaps the old question as to the storing of oxygen in cells must be reopened again. One should like to say, "Without oxygen neither life nor light." Such an epigram can only be true when, by oxygen, we refer to the element both free and combined. If we speak of anaërobic existence, we must also recognize anaërobic luminescence.

I arrived at this discovery through the observation that, although the luminescence of *Cypridina* is inhibited by strong light in presence of oxygen but not in its absence, the luminescence of *Mnemiopsis* and other Ctenophores is inhibited by light whether oxygen be present or not. This led me to reinvestigate methods of removing oxygen from fluids and the discovery that Ctenophore extracts can still luminesce under anaërobic conditions where other luminous forms (including *Cypridina*) show no trace of luminescence. It seems very probable, then, that in Ctenophores oxygen is firmly bound and that this is the reason we observe inhibition of their luminescence by light in absence (apparent) of oxygen. If oxygen is not bound in Ctenophores, their luminescence must result from a totally different type of reaction from that in other forms, for I believe the means employed for oxygen removal are without criticism.

[1] Appointed to the A. A. A. S. table at Naples, Oct. 1925–Jan. 1926.

There are four convenient methods of removing the last trace of oxygen from biological fluids. I say methods for *biological* fluids because none of these methods involves very great changes in H-ion concentration that might bring about changes in the fluid, although all of them are not suited for living cells. All these methods prevent the luminescence of bacteria (Harvey and Morrison, 1923) and *Cypridina*, which certainly means less oxygen than 10^{-5} atmospheres, and most of them involve nascent hydrogen.

1. By addition of cells actively using oxygen such as muscle, yeast or bacteria. If the fluid is a cell extract requiring oxygen, it will use up its own oxygen on standing in a narrow tube. The reducing power of cells varies but practically all will reduce methylene blue and most will reduce indigo-carmine. For the significance of this in terms of oxidation-reduction potential the reader is referred to the papers of Clark (1923–25).

2. Nascent hydrogen can be generated in the fluid by adding aluminium amalgam or magnesium plus some neutral ammonium salt. In the first case $Al(OH)_3$ is formed and in the second an ammonium-magnesium salt. In either case the P_H does not change much, and the oxygen is quickly washed out.

3. Small amounts of sodium hydrosulphite (or hyposulphite, $Na_2S_2O_4$, not thiosulphite) may be added to the fluid. It is best to dissolve the hydrosulphite in a powerful buffer and to buffer the fluid under investigation as the hydrosulphite is acid. To determine when the right amount of hydrosulphite has been added methylene blue can be used as indicator. It is decolorized when reduced by the hydrosulphite, which also absorbs all oxygen in the solution. Shaking with air or addition of potassium ferricyanide [$K_3Fe(CN)_6$] will oxidize the hydrosulphite with return of the blue color to methylene blue. The advantage of hydrosulphite is its practically instantaneous production of anaërobic conditions. The disadvantage is a possible injurious effect on living cells, which I believe should be investigated rather carefully. My own observations show that the ciliated cells of the gills of *Mytilus*, lightly stained in methylene blue, stop beating and the blue color disappears (reduction) in hydrosulphite sea water very quickly. When fresh aërated sea water

is added the blue color returns and then the cilia begin beating again.

The luminescence of a mixture of *Cypridina* luciferin and luciferase is immediately extinguished by hydrosulphite and returns again on shaking with air at a time when methylene blue is just beginning to show a slight blue color. If some dry powdered *Cypridinas* are added to hydrosulphite sea water, it will be observed that fragments of undissolved luminous material are luminescent but the light disappears very quickly. There is apparently some air entangled, possibly mechanically, with the dry photogenic substances and when this is used up luminescence ceases. I had previously made this observation in another manner (Harvey, 1920).

In using methylene blue as an indicator, it must be borne in mind that mere removal of oxygen will not reduce methylene blue but that once reduced, a blue color will return when traces of oxygen are present. However, the reoxidation of methylene blue is rather slow except in alkaline solutions. The color change of methylene blue is also affected by light (Clark, 1925). Absence of luminescence in *Cypridina* luciferin-luciferase or in luminous bacteria can also be used as an indicator of lack of oxygen (the former is more delicate; bacteria begin to luminesce at an oxygen pressure = .0053 mm. Hg) and mere removal of oxygen is sufficient for cessation of luminescence in both cases.

4. Addition of platinized asbestos to the fluid and passage of hydrogen through it. This affords a quite rapid method of removing oxygen from fluids and the hydrogen need not be absolutely free of oxygen. It is far more rapid than bubbling pure hydrogen through the fluid without platinized asbestos. By pure hydrogen I mean hydrogen that has been passed over platinized asbestos heated to dull redness in a quartz tube. Such hydrogen contains oxygen in equilibrium at 10^{-26} atmospheres. It must not come in contact with rubber or oxygen will be absorbed from the rubber in sufficient amount to cause luminescence of *Cypridina* luciferin-luciferase solution. My studies on luminescent solutions have impressed me with the difficulty of removing small amounts of oxygen from fluids and the slowness with which a fluid comes into equilibrium with a

gas. I believe the addition of platinized asbestos to the fluid through which hydrogen is passed will prove of great value to biologists in the study of cells under anaërobic conditions.

Instead of Pt asbestos, colloidal Pt or colloidal Pd may be used. The latter is conveniently prepared by adding to 5 cc. 0.2 per cent. $PdCl_2$ just enough dilute NaOH to make the $PdCl_2$ darken without giving a precipitate of basic $PdCl_2$, and then one drop of .042 per cent. hydrazine hydrate. The finely divided Pd adsorbs a good deal of hydrogen and will keep a solution free of oxygen for some time, as can be observed by using as indicator the luminescence of a mixture of luciferin and luciferase.

In order to show that the luminescence of Ctenophores, like *Beroë*, is independent of dissolved oxygen, it must be recalled that an extract made by squeezing *Beroë* through muslin and filtering through filter paper is non-luminescent itself, but gives a brilliant light on addition of fresh water, ***dilute*** salt solution, or various cytolytic substances like saponin, sodium glycocholate, ether, etc. Accordingly I tried the experiment of mixing *Beroë* extract made free of oxygen by $Na_2S_2O_4$ with $m/10$ Na_2HPO_4 solution, also made free of oxygen by $Na_2S_2O_4$. The Na_2HPO_4 was used instead of fresh water in order that the reaction would be alkaline enough to allow rapid coloration of leuco-methylene blue, added as an indicator, should any oxygen leak into the apparatus. This is shown in Fig. 1. In *A* is placed *Beroë* extract + $Na_2S_2O_4$

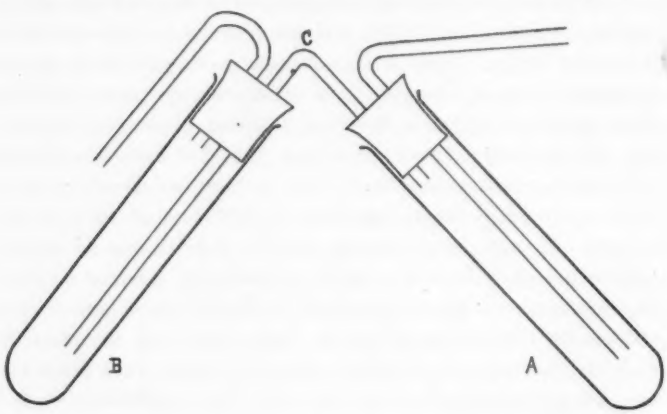


FIG. 1.

and in *B* $m/10$ Na_2HPO_4 solution ($P_H = 9$) + $Na_2S_2O_4$. The tubes allow a flow of pure hydrogen through a lead tube to wash out the air, and the only rubber parts are the stoppers of tubes *A* and *B*. In such an apparatus the contents of *A* and *B* can be mixed in hydrogen without the slightest blueing of leuco-methylene blue and oxygen-free *Cypridina* luciferin can be mixed with oxygen-free luciferase (by methods 3 or 4) without any luminescence appearing. However, when the oxygen-free *Beroë* extract (by methods 3 or 4) is mixed with the oxygen-free $m/10$ Na_2HPO_4 solution, just as bright a luminescence occurs, as in the control tests when mixed in presence of oxygen.

The experiment has been varied by using Pt asbestos in the *Beroë* extract and in the water and passing pure hydrogen through the system for an hour (although methylene blue can be decolorized in two or three minutes by the same flow of hydrogen), but a bright luminescence always results on mixing the *Beroë* extract with the water in the hydrogen atmosphere. When air is admitted and the fluid shaken, no further luminescence is to be observed.

The experiment has been repeated so often that I feel certain that Beroë extract will luminesce in absence of dissolved oxygen. *Eucharis multicornis*, another Ctenophore, behaves as *Beroë*, as does also the medusa, *Pelagia noctiluca*. *Pennatula phosphorea* extract, on the other hand, requires oxygen, as I had previously observed for the pennatulid, *Cavernularia haberi* Harvey (1917). A *Pennatula* extract in sea water mixed with fresh water in absence of oxygen [1] gives no light but when shaken with air the mixture luminesces faintly. It is somewhat surprising that the pennatulids should differ in this respect from the Ctenophores and *Pelagia* as the general aspects of the luminescence in these forms is similar.

None of these animals give the luciferin-luciferase reaction. The luminescence seems to be connected with the dissolution of granules. In the luminous slime of *Pelagia*, which sticks to the finger when rubbed over the exumbrella, can be observed with the microscope, cells densely crowded with granules somewhat less than 1 μ in diameter. These granules and some cell frag-

[1] Mixing in air of course results in luminescence.

ments will pass filter paper and centrifuging such a filtrate will throw down the cell fragments and many granules, leaving suspended only granules. When added to fresh water this centrifuged material gives a bright luminescence (because many granules are thrown down) while the suspension above gives a fainter luminescence. I could observe no cells in the suspension but when mixed with fresh water in complete absence of dissolved oxygen (method No. 4, hydrogen passed 20 mm.), as bright a luminescence occurred as when oxygen was present. I therefore believe that the oxygen is bound in these photogenic granules and that luminescence occurs upon their dissolution. That just enough or more than enough oxygen is so bound would be indicated by the fact that opening the tube containing the mixed fluids to the air results in no further luminescence.

This same experiment has been repeated with filtered and centrifuged *Beroë* extract, with the same result, luminescence of granular material in absence of oxygen.

The Radiolaria also require no free oxygen for luminescence. *Thalassicolla nucleata* and also colonies of *Colozoun inerme* mixed with platinized asbestos in sea water through which pure hydrogen is passed for 45 minutes will still luminesce whenever shaken. A colony of *Colozoun inerme* was broken up into separate cells (but these left uninjured) mixed with platinized asbestos and pure hydrogen passed through it for 20 minutes. It was then mixed in a pure hydrogen atmosphere with water and platinized asbestos through which pure hydrogen was passed for 20 minutes. A bright "starry" luminescence resulted, similar to that of *Beroë* extract when mixed with water.

After these rather startling (to me) results I thought it worth while to reinvestigate the necessity of oxygen for luminescence in other animals, noting carefully if the lack of oxygen caused luminescence to disappear when the whole animal was tested and when its secretion was tested. For we are especially interested in knowing whether the luminescence of dissolved or finely divided photogenic material is stopped by lack of oxygen rather than whether intact cells cease to luminesce in absence of oxygen. It is of course perfectly well known that luminous bacteria cease to luminesce in absence of oxygen but one could not make the

same statement of the *extract* of luminous bacteria for luminous material cannot be obtained apart from the living bacterial cell. The same is probably true of luminous fungi. They (*Panus stipticus*) require oxygen for luminescence. If ground in a mortar, *Panus stipticus* luminescence ceases almost immediately, so that an extract cannot be obtained.

The work of many investigators has shown that luminous *extracts* of *Pholas* (lamellibranch mollusc), *Cypridina* (ostracod crustacean), *fireflies*, *Cavernularia* (pennatulid), *Photoblepharon*,[1] *Anomalops*,[1] and *Malacocephalus* (fish) require oxygen for luminescence. I have recently investigated the following forms using extracts of the animals whenever possible.

Microscolex phosphorea, an earthworm producing a slime on stimulation, brightly luminescent with a yellowish light. The worm can be dried over $CaCl_2$ and gives a bright light on moistening with water. Worms shaken in pure hydrogen give no luminescence but produce a slime which luminesces immediately when air is admitted.

Chætopterus variopedatus, a polychæte producing a slime with bluish light on stimulation. In sea water the luminescence of the slime lasts long enough for one to observe the disappearance of luminescence when pure hydrogen is bubbled through it and the reappearance on admitting air.

Acholoë astericola, a polynoid worm whose scales emit a bright yellowish luminescence on stimulation. The light comes from groups of gland cells under the cuticula ending in a papilla with a pore, but not enough secretion is extruded to form a luminous solution. The whole animal when slowly heated in sea water or shaken gives a bright luminescence (over the scales) but if slowly heated or shaken in sea water with platinized asbestos through which hydrogen is passed, there is no luminescence.

Thelepus cincinnatus, a polychæte allied to *Polycirrus*, produces a luminescent slime on handling. When the whole worms are placed in a tube through which pure hydrogen is bubbled the luminescence disappears, but returns again when air is admitted.

Amphiura squamata, a brittle star whose arms produce a yellowish luminescence. The individual gland cells lie beneath

[1] The light of these forms is due to symbiotic luminous bacteria living in the organ.

the surface of certain plates and possess ducts that empty through pores in the cuticula, but not enough secretion is passed to the outside to give a luminous solution. The whole animals stimulated electrically in sea water plus platinized asbestos and air give a bright luminescence but no luminescence when stimulated in sea water plus platinized asbestos through which hydrogen has been passed for some time.

Balanoglossus minutus.—The whole of the skin produces a luminescent slime whose light does not last very long. If the whole animal is shaken in sea water through which pure H_2 is passing, there is no luminescence but on admitting air both worm and sea water show luminescence.

Copepods.—(Several species.) The cells are unicellular glands in definite positions in the body or in the legs, secreting to the exterior. When heated slowly in sea water with air the copepods give a bright luminescence but when heated slowly in sea water and platinized asbestos through which hydrogen was passed there was no luminescence. In one experiment the temperature was raised to boiling in the hydrogen atmosphere, then cooled and air admitted. The *solution* became faintly luminescent. A small amount of oxygen will cause luminescence and the hydrogen must be passed for some time to obtain anaërobic conditions.

Heteroteuthis dispar.—A squid in which a secretion from a gland (part of ink sac) is shot into the sea water as a bluish luminescent cloud of small granules often sticking together in clumps and filaments. On a microscope slide under a cover slip the secretion luminesces only at the edges in contact with air (but luminesces uniformly when the cover slip is lifted). When hydrosulphide is added to the secretion in a test tube the luminescence disappears except at surface but returns on shaking with air. Oxygen is necessary for luminescence.

Quatrefages observed luminescence of *Noctiluca* under what he regarded as anaërobic conditions whereas E. B. Harvey (1917) found the luminescence of *Noctiluca* to become very faint in a current of impure hydrogen (made in a Kipp generator but last traces of oxygen not removed) but to regain its brightness when air was admitted. It would seem that *Noctiluca* must require oxygen for luminescence. In view of my experiments with

Radiolaria and Cœlenterates it would certainly be worth while to test the luminescence of dinoflagellates, hydroids, siphonophoræ and medusæ in absence of oxygen. Many other higher forms await careful experimental investigation.

SUMMARY.

Simple methods for completely removing oxygen from biological fluids are described and indicators for absence of oxygen discussed.

Most luminous animals require free gaseous dissolved oxygen for luminescence but a few can luminesce without such oxygen. These are the Ctenophores; the medusa, *Pelagia noctiluca;* and Radiolarians. Pennatulids require oxygen as do all annelida, ophiurians, cephalopoda, copepoda, and balanoglossids tested.

It is found that in *Beroë* and *Pelagia* the photogenic granules (without cells) luminesce in absence of oxygen, and it is suggested that the proper amount of oxygen for luminescence is bound up in the photogenic granule, and cannot be removed by the drastic methods of oxygen-removal herein described.

LITERATURE.

Clark, W. M.
'23–'25 Public Health Reports, Washington, especially XL., 1139.
Harvey, E. N.
'17 Amer. Journ. Physiol., XLII., 349.
Harvey, E. N.
'20 Amer. Journ. Physiol., LI., 580.
Harvey, E. B.
'17 Carneg. Inst. Wash., Pub. No. 251, p. 244.
Harvey, E. N., and Morrison, T. F.
'23 Journ. Gen. Physiol., VI., 13.

COMPLETE SEX-REVERSAL IN THE VIVIPAROUS TELEOST *XIPHOPHORUS HELLERI*.

J. M. ESSENBERG,

ANATOMY LABORATORY, SCHOOL OF MEDICINE,
UNIVERSITY OF OKLAHOMA.

I. INTRODUCTION.

During the progress of the study on sex-differentiation of *Xiphophorus helleri* it became apparent that sex was not a fixed factor in this species. It was found (Essenberg, 1923) that 50 per cent. of the young females transformed to males before sexual maturity was reached. There were also indications that sex-reversal is not confined to immature fishes but that it may occur in adult specimens which have previously born young. To test the merits of such indications experimental plans were conceived which, together with the results, are considered in the present paper.

With the most favorable environment which prevailed at the Hull Zoölogical Laboratory and the abundance of *Xiphophorus helleri*, it was relatively an easy matter to initiate experiments. A large number of adult females were isolated into separate aquaria for observation. Close watch of these specimens was kept for more than a year but with totally negative results.

In the fall of 1922, the writer became associated with the Medical School of the University of Missouri, where the work was to be continued. Early in the spring of the following year a shipment of fish was received from the University of Chicago, for which I wish to express my gratitude to Dr. A. W. Bellamy, and another shipment was procured from the Crescent Fish Farm of New Orleans. Fishes of both shipments were mixed before they were isolated into separate aquaria. Strict records of each isolated fish were kept. The records were to show the date of isolation, date of birth of young, and the number of young at each birth.

At the beginning of July, 1923, one of the adult females which

had given birth to forty young on the second of June and fifty-three on the fourth of May failed to bring forth young. It is necessary to state here that the species under consideration reproduces monthly with almost chronological regularity. At the time mentioned there were no definite signs of sex-reversal and it was thought that owing to some change in environment, birth of young was delayed. A month later, that is the beginning of August, signs were available as to what was going on with female B16. September the 12th, when the writer left for Norman, Oklahoma, to occupy his present position, the fish had reached advanced stages of sex-reversal.

Owing to the courtesy of Dr. G. W. Tannreuther of the Department of Zoölogy, to whom I wish to express my indebtedness, my research material reached Norman in good shape a few weeks later. Female B16 was an unmistakable male except body form by the first of December and was mated to a virgin female. On February 25, 1924, this pair gave birth to eight young, all of which were to all appearances normal. Two weeks later five of the young fish died owing to a sudden drop of temperature. The remaining three reached sexual maturity with one male and two females as a sex-ratio.

The stock was greatly increased in the spring of 1924 when additional material was procured from the Crescent Fish Farm. A larger number than ever before of adult females, not less than three years of age, were isolated for observation. Of this group three females transformed to males before the end of the year. One, female C32, transformed without previously giving birth to young and for this reason is dismissed from further consideration in this study. Female C3 gave birth to one litter and female C14 three litters of young before sex-reversal took place. Both fishes were mated to virgin females, but young were obtained only from the mating in which C3 was the male. From this mating five young were obtained, all of which reached sexual maturity. The sex-ratio was two males to three females.

As stated above, in the mating in which C14 was the male no young were obtained. The mating was repeated with other virgin females but without success. Upon killing, the autopsy revealed malformation of the sperm duct which was obstructed

at its anterior end. The testes were well formed and contained abundant spermatophores.

For convenience of description the process of sex-reversal in *Xiphophorus helleri* may be divided into three, more or less, definite stages. The first ranges from a normal female to the enlargement of the anal fin, the second includes the formation of the gonopod, and the third is concerned with the development of the "sword" in the tail fin.

II. First Stage in Sex-reversal.

The first indication of sex-reversal in these fishes is the absence of the regular monthly delivery of young. To be sure, there are other factors which inhibit reproduction such as lack of fertilization, certain temperatures, light, and composition of the water, but these are exceptions and can be eliminated in normal and well-balanced aquaria. With the failure of reproduction the observer's attention is focused on other landmarks in the history of sex-reversal, the most important of which is a characteristic black area known as the puberty spot.

The puberty spot is found in all sexually mature females and is located just above the pelvic fin. Its size varies with the age of the fish or rather with the size of the ovary. In young virgin females it is less than one fourth of an inch in diameter, whereas in females of three years of age it approaches half an inch in diameter. The puberty spot is caused by the black pigment visible through the body wall which accumulates in the peritoneum adjacent to the ovary. With onset of sex-reversal the black pigment in the peritoneum gradually fades and in the course of six to eight weeks the puberty spot has entirely disappeared.

Sex colorations in *Xiphophorus helleri* exhibit very little, if any, dimorphism. During sex-reversal the bright red and black pigments of the fins and lateral line dim to such an extent that the fish becomes, so to speak, "invisible." This appearance of the animal is retained until the gonad of the opposite sex has begun to grow and is capable of producing a certain amount of the male sex hormone.

The internal changes which take place during this stage are

significant and must, to a certain extent, be reviewed here. For further details of description and figures on sex-differentiation the reader is referred to Essenberg (1923).

As far as the writer knows, the causative factor or factors in sex-reversal have not been successfully demonstrated. That these factors reside in the gonad and possibly in the germ cells themselves has been substantiated beyond a doubt by experimental data. Whenever those factors become functional the entire ovary is subject to hopeless degeneration. At first the large ovocytes disintegrate, then the medium-sized ones, and finally the young ovocytes which are found close to the epithelium of the ovarian cavity follow their two predecessors. Also the epithelium covering the ovary, which originates from the peritoneum of the body cavity, disintegrates completely. The epithelium of the ovarian cavity, however, is not subject to disintegration. It shrinks and assumes more or less a tubular form, its cells, judging from their appearance, undoubtedly undergo retrogressive changes but no disintegration is noticeable. After a period of rest this epithelium becomes active and proliferates cells which go to form the gonad of the opposite sex—the testis. The process of testicular formation which belongs to the present stage of sex-reversal includes the proliferation of germ cells from the remains of the epithelium of the ovarian cavity and the formation of sex-cords. It includes two stages of sex-differentiation called by the writer "Early and Middle Stages of Tubule Formation" (Essenberg, 1923, pp. 57–58).

It is interesting to note that the epithelium of the ovarian cavity which gives rise to the definitive male cells originates, as does the epithelium covering the ovary, from the peritoneum of the body cavity.

While the process of disintegration is taking place, the vitality of the transforming animal is reduced to the minimum. Voluntary motion which is so characteristic of *Xiphophorus helleri* is seldom noticed; the fish rests most of the time and moves only to escape danger or because of hunger. As to the latter it may be said that the animal takes very little food of any kind during the process of ovarian disintegration. This is a critical stage to the animal. The least change in the animal's environment,

such as slight fluctuation in temperature, water composition or disturbance will inevitably result in the death of the fish. It seems probable that sex-reversal in *Xiphophorus helleri* is not preceded by the increase of metabolic rate but that the opposite is true. Nor is it true that the transformation is caused by tuberculosis or any other kind of disease as far as it can be detected either macro- or microscopically.

III. Second Stage in Sex-reversal.

The second stage in sex-reversal deals with the transformation of the anal fin of the female into a functional intromittent organ or gonopod and with the parallel development of the gonad.

The anal fin of the female consists of ten pairs of rays all of which are approximately of the same length and diameter. The above statement applies equally well to the anal fin of young fishes during the indifferent stage of development. During sex-differentiation in the male direction or sex-reversal, which in *Xiphophorus helleri* is always from female to male, several pairs of the rays undergo a peculiar metamorphosis to form a copulatory organ. The first sign of such transformation is the thickening of the third pair of rays. While this is in progress, the third, fourth, and fifth pairs of rays elongate until approximately twice the length of the original fin is reached. The first, second, and sixth to tenth pairs of rays are subject to no special changes and remain rudimentary. The third pair reaches a thickness several times its original diameter and serves as a supporting bar or rod of the gonopod. The apex of this pair of rays together with the fourth and fifth pairs form hooks and counterhooks which serve the purpose of anchorage to the female genital pore during copulation. It is not a hollow penis-like structure but a solid bar which acts as a guide in transmitting of spermatophores from male to female genitals.

The development of the gonad during the second stage of sex-reversal corresponds to the " Late Stage of Tubular Formation " in sex-differentiation. The solid sex-cords which were formed during the first stage acquire a lumen and become branched and subbranched. The cells constituting the sex-cords or tubules, which thus far were not unlike their sister cells of the

peritoneum undergo characteristic transformation. The cytoplasm as well as the nucleus increases in size and assumes a spherical form. When these cells have completed their differentiation they can not be distinguished microscopically from the primordial germ cells of the indifferent stage.

IV. The Third Stage in Sex-Reversal.

The third stage in sex-reversal is characterized externally by the development of a "sword"-like process in the tail fin and internally by spermatogenesis.

It is true that there is a slight overlapping between the second and third stages, yet it is desirable to consider these separately for the reason that they constitute definite landmarks in the process of sex-reversal.

The sword of the caudal fin begins to develop slightly before the gonopod reaches its completion. It is formed from the ventral lobe of the tail fin. The ten ventral rays of this fin are involved. Most of the elongation falls upon the sixth to the tenth and the utmost length is reached by the eighth ray. The length of the fully formed sword approximates the total length of the fish. It is abundantly supplied with brilliant colors and its sole purpose seems to be display.

The development of the testis in this stage of sex-reversal consists of spermatogenesis. A number of germ cells develop in a constricted portion of the sex-cord or tubule and form an acinus or spermatocyst. Every cell in such a cyst is in the same stage of development from the beginning to the end and they ultimately form the spermatophore. At the end of the so-called "Middle Stage of Tubular Formation," the germ cells are primary spermatocytes. The secondary spermatocyte stage is reached by cell division which doubles the number of germ cells and the volume of the acinus. With another cell division which doubles the cell number but not volume of the acinus the germ cells reach the spermatid stage. With the metamorphosis of spermatids into spermatozoa the acinus which thus far has been so tightly packed with germ cells that it simulates a syncytium now becomes luminated. The lumination owes its origin to the fact that the spermatozoa move towards the

periphery of the acinus and form a lining to the thin membranous wall of the acinus. The lining consists of only one layer and is formed by the nuclei or heads of the spermatazoa. The tails project freely into the lumen. With this the acinus has metamorphosed into the mature spermatophore which during copulation reaches the female genitals by the aid of the gonopod.

V. Discussion.

Fertility before Sex-Reversal.

To eliminate the chance of error and prevent misunderstanding, it was decided that only those females which are definitely known to have produced young will be considered in the study of sex-reversal. The records of female B16 shows that she gave birth to 53 young on the 4th of May, and to 40 young on June the 2d, 1923. The records of female C3, which are equally complete, show that only one litter of young were born before she began to change in the male direction. If it is remembered that the females of *Xiphophorus helleri* reach sexual maturity before they are one year of age, and that the fishes were at least three years of age before they came under the writer's observation, it is seen that females B16 and C3 have been experiencing the instincts of motherhood not less than two years before they experienced the instincts of fatherhood.

Fertility after Sex-Reversal.

From the mating in which B16 was the male, eight young were born. C3 became the father of five young, all of which developed to normal, sexually mature fish. It can be stated that the mortality in the progeny of B16 was wholly due to the inability to control temperature in the aquaria. The sex ratio of the young obtained compares favorably with sex ratios found by the writer in his study of sex-differentiation (1923).

The Origin of Testicular Tissues.

It has been shown (Essenberg, 1923) that the definitive germ cells of *Xiphophorus helleri* are not linear descendants of primordial germ cells but originate from peritoneal epithelium. The primordial germ cells either entirely disintegrate (females) or

become segregated and thus never take part in definitive germ cell production (males). The epithelium of the ovarian cavity which gives rise to definitive ovocytes proliferates cells which go to form the testes of the transforming fish. The epithelium of the ovarian cavity originates in the following fashion. As the primordial germ cells of this teleost project into the body cavity, they are covered by the peritoneal epithelium. During the indifferent stage the gonads are paired. If the young fish develop in the male direction, the paired or bilateral relation is retained permanently. In case the animal develops into a female, the undifferentiated bilateral gonads fuse medially in such a manner that the medial parts of the covering of the gonads form the epithelium of the ovarian cavity. In the course of sex-reversal the entire ovary disintegrates except the epithelium of the ovarian cavity. From it cells proliferate which form sex cords and later seminiferous tubules.

Proof to the effect that the same is true in birds was furnished by the excellent work of Fell (1923). This author had the unique opportunity to study the gonads of eight arrhenoid birds which represented the various stages in sex-reversal from female to male. It was found that the germ cells of the opposite sex proliferate from the peritoneal epithelium of the degenerating ovary as well as from the peritoneal epithelium of the vestigial right ovary. These cells form sex cords which in due time give rise to seminiferous tubules. The sperm cells from such tubules are known to be functional. The author concludes that "we have here a case of direct transition of ordinary somatic peritoneal cells into germ cells."

Similar observations have been made by Gatenby (1916), Firket (1920), and by Swingle (1921). In the light of our present knowledge, it may indeed be questioned if definitive germ cells ever are lineal descendants of primordial germ cells in higher animals.

It has been claimed that the peritoneal epithelium covering the ovary is "packed" with primordial germ cells which have reached the epithelium by migration and that those are the primordia of sex cord formation. Whatever might be the validity of this claim in case of birds the writer is not in position

to say, but merely wishes to point out that the claim is inapplicable in case of *Xiphophorus helleri*. The primordial germ cells reach the body cavity en masse by passive displacement brought about by developmental processes of adjacent musculature and at no time do germ cells fuse with cells of peritoneal epithelium.

Causes of Sex-Reversal.

A case of complete sex-reversal in ring dove was described by Riddle (1923). The autopsy revealed that the viscera of the bird were seriously affected by tuberculosis which, together with other cases observed by Riddle, led him to the conclusion that tuberculosis is the immediate cause of sex-reversal. Based on the fact that the male bird has a higher metabolic rate than the female, Riddle assumes that the function of tuberculosis is to increase the metabolic rate of the diseased female bird. When once the metabolic rate has been increased, the diseased animal, according to Riddle, has nothing to prevent it from acquiring the anatomy and physiology of the opposite sex.

The data gathered from fishes undergoing sex-reversal do not seem to favor the claim that there is an increase of metabolic rate prior to, or during, transformation of sex. Indeed there are definite indications that the opposite is true. The transforming fishes become very inactive, probably take no food during early stages and are incapable of adapting themselves to changing environment even if the change be very slight.

Another case of complete sex-reversal in birds was describe by Crew (1923). This bird, a Buff Orpington hen, was als affected with tuberculosis of the viscera. Crew claims that sex-reversal is preceded by some disease such as tuberculosis, hemorrhage, tumor growth, etc. The disease affects the ovarian secretion and the general metabolism of the bird in such a way that the conditions favorable for the differentiation and growth of spermatic tissue are created.

It is definitely known that the fishes described in this paper were not diseased, certainly not by tuberculosis. Every transformed fish has been sacrificed to establish this very point. A number of diseased fishes have been observed, but none of these have become subject to sex-reversal. A case of complete

sex-reversal in the domestic fowl is described by the writer (1926) in which no disease whatever is discovered. From the above fact the conclusion is warranted that disease is not a necessary precursor of sex-reversal.

It seems unquestionably true that the factor or factors which determine and control sex must also determine and control sex-reversal. It seems equally true that such factor or factors are of labile rather than fixed nature.

The chromosome theory has been considered as an adequate explanation of sex determination and control. It is assumed that sex is determined at fertilization at which the zygote receives the male or female constitution which it maintains for life.

In the light of data accumulated for such studies as hermaphrodites, intersexes and particularly from cases of complex sex-reversal, the chromosome theory seems to have reached the limit of flexibility. It is difficult to see how a definite chromosome composition which determines and controls sex can be completely overridden and still remain as a sex-controlling mechanism. The inadequacy of the chromosome theory finds expression in the often reiterated statement that "sex is not irrevocably decided by the sex-chromosome constitution." It seems logical to expect that there must be something that does decide sex and possibly the 'sex-chromosomes.'

Proof that maleness and femaleness depends upon the secretions of the testes and ovary has been supplied by a number of investigators. Perhaps the clearest cut data have come from castration and transplantation experiments, particularly from the work of Zawadowsky (1922). This author finds that after a total castration of a young cockeral he loses his voice, sex instincts, and head-furnishings and thus assumes an asexual or generic appearance. However, if an ovary is successfully implanted into such castrated male, it will not only assume the appearance of the hen but the graft produces ovocytes as well. In case a young hen is castrated, she will acquire cock-feathers and spurs after the first molting. If however, the castration is incomplete, and the ovary has the chance to regenerate, the bird regains its female furnishings. Hens with total removal of ovary, develop testes as well as external fur-

nishings of the male within four to five months. Castrated hens with implanted testes acquire male furnishings and if the transplantation is successful, the graft produces active spermatozoa.

From these and other experiments of similar nature which he has carried on with mammals, Zawadowsky concludes as follows: "Aus meinen Versuchen bin ich geneigt Hinweise darauf zu ersehen, dass hinter den Symbolen der 'Geschlechtsgene' F (female) und f (male) die geschlechts hormonen- Feminisin und Masculinisin zu suchen sind."

With the above statement the present writer is in full accord and wishes to add that with the replacement or substitution, if you please, of feminisin or the female hormone for the gene-anatomy represented by X and masculinisin or male hormone for the qualities of Y, the problems of sex are brought in accord with present knowledge of bio-chemistry and endocrinology. By so doing we not only gain an insight into the mechanics of the various types of hermaphrodite, of "intersex," and sex-reversal cases, which thus far have been a source of puzzle and speculation, but we also gain a better understanding of the science of genetics.

In conclusion, it may be said that there is no particular disease associated with sex-reversal. Any adverse condition be it extrinsic or intrinsic, acquired or inherited, which tends to reduce the capacity for hormone production beyond a certain limit may automatically become the immediate cause in sex-reversal in the female of *Xiphophorus helleri*.

VI. Summary.

1. Two cases of complete sex-reversal, from female to male, in adult *Xiphophorus helleri* have been described.

2. It is definitely known that both fish gave birth to normal young prior to sex-reversal.

3. It is also definitely known that both fish fertilized virgin females which gave birth to young with sex-ratio typical of the species.

4. The transformed fish is indistinguishable from a "normal" male except body form which is that of a female.

5. The entire ovary disintegrates except the epithelium of the

ovarian cavity. From it cells proliferate and form sex cords in sex-reversal cases.

6. The epithelium of the ovarian cavity is derived from the peritoneal epithelium and at no time is infiltrated with primordial germ cells.

7. The position, structure and physiology of the testis of the transformed fish is identical to that of the "normal" males. The oviduct becomes the spermduct.

8. No tubercular lesions or lesions of any other disease were found in the arrhenoid fishes.

9. Chromosomes are not considered as having the function of sex determination and control.

10. Sex is determined and controlled by the sex hormones derived from the ovary and testes.

11. Any agent or condition which tends to decrease the capacity for hormone secretion becomes an immediate factor in sex-reversal.

BIBLIOGRAPHY.

Blacher, L. J.

'26 The Dependence of Secondary Sex-characters upon Testicular Hormones in *Lebistes reticulatus*. BIOL. BULL. (In press).

Champy, M. Ch.

'21 Changement experimental du sexe chez le *Triton alpestris* Laur. De L'acad. des Sci., Tome 172.

Chidester, F. E.

'17 Hermaphroditism in *Fundulus heteroclitus*. Anat. Rec., Vol. 12, p. 389.

Crew, F. A. E.

'21 Sex-reversal in Frogs and Toads. A review of the recorded cases of abnormality of reproductive system and an account of a breeding experiment. Jour. Genetics, Vol. 11.

Crew, F. A. E.

'23 Studies in Intersexuality. Part II. Sex-reversal in the Fowl. Proc. Roy. Soc., B. Vol. 95, p. 256.

Eigenmann, C. H.

'96 Sex-Differentiation in the Viviparous Teleost *Cymatogaster*. Arch. f. Entw. Med., Bd. IV, p. 125.

Essenberg, J. M.

'23 Sex-Differentiation in the Viviparous Teleost *Xiphophorus helleri* Heckel. BIOL. BULL., Vol. 45, p. 46.

Essenberg, J. M.

'26 Complete Sex-Reversal in the Domestic Fowl. Anat. Record (In press).

Fell, Honor B.

'23 Histological Studies on the Gonads of the Fowl. I. The Histological Basis of Sex-Reversal. Brit. Jour. Exp. Biol., Vol. 1, p. 97.

Firket, Jean.

'20 On the Origin of Germ Cells in Higher Vertebrates. Anat. Rec., Vol. 18.

Geiser, S. W.

'21 Observations on Sex in the Top-minnow, *Gambusia afinis*. Proc. Toronto Meeting, Anat. Rec., Vol. 23.

Goldschmidt, R.

'16 Experimental Intersexuality and the Sex Problem. Am. Nat., Vol. 50, p. 705.

Kopéc, Stefan.

'18 Contribution to the Study of the Development of the Nuptial Color of Fish. Sprawozdania Taw. Nauk, Warsz., Rok. 11, 1918, Zesgyt, 1.

Lillie, Frank R.

'17 The Free-Martin; A Study of the Action of Sex Hormones in the Fœtal Life of Cattle. Jour. Exp. Zoöl., Vol. 23, p. 371–453.

Moore, Carl R.

'21 On the Physiological Properties of the Gonads as Controllers of Somatic and Psychical Characteristics. III. Artificial Hermaphroditism in Rats. Jour. Exp. Zoöl., V. 33, p. 129.

Newman, H. H.

'08 A Significant Case of Hermaphroditism in Fish. BIOL. BULL., Vol. 15.

Okkelberg, Peter.

'21 The Early History of the Germ Cells in the Brook Lamprey, *Entosphenus Wilderi* (Gage), up to and Including the Period of Sex-Differentiation. Jour. Morph., Vol. 35.

Philippi, Erich.

'04 Ein neuer Fall von Arrhenoidie. S. B. Ges. naturf. Freunde, Berlin.

Riddle, Oscar.

'24 A Case of Complete Sex-Reversal in the Adult Pigeon. Amer. Nat., Vol. 58, p. 167.

Sand, Knud.

'19 Experiments on the Internal Secretions of the Sexual Glands, Especially on Experimental Hermaphroditism. Jour. Phys., Vol. 53, p. 257.

Sellheim, Hugo.

'25 Vermännlichung und Wiederverweiblichung bei einem ausgewachsenen Individuum. Zeitsch. Micro.-Anat. Forsch., Bd. 3, p. 382.

Southwell, Thomas.

'02 On a Hermaphrodite Example of the Herring (*Clupea harengus*). Am. Mag. Nat. Hist., Vol. 9, pp. 195–196.

Steinach, E.

'20 Künstliche und natürliche Zwitterdrüsen und ihre analogen Wirkungen. Archiv. f. Entw.-Mech., Bd. 46, S. 12.

Swift, C. H.

'14 Origin and the Early History of the Primordial Sex Cells in the Chick. Am. Jour. Anat., Vol. 20.

Swingle, W. W.

'17 The Accessory Chromosome in a Frog Possessing Marked Hermaphroditic Tendencies. BIOL. BULL., Vol. 33.

Swingle, W. W.

'21 The Germ Cells of Anurans, I. The Male Sexual Cycle of Rana Catesbeiana Larcæ. Jour. Exp. Zoöl., Vol. 32.

Swingle, W. W.

'26 The Determination of Sex in Animals. Physiol. Review, Vol. 6, p. 28.

Witschi, Emil.

'21 Chromosomen und Geschlecht bei Rana Temporaria. Deutsche Ges. f. Vererb., Bericht Aug. 1921, p. 24.

Witschi, Emil.

'21 Der Hermaphrodismus der Frösche und seine Bedeutung für das geschlechtsproblem und die Lehre von der inneren Sekretion der Keimdrüsen. Arch. f. Ent.-Med., Bd. 49, p. 316.

Zawadowsky, M.

'22 Das Geschlecht und die Entwickelung der Geschlechtsmerkmale. Moscow (State edition).

SCROTAL REPLACEMENT OF EXPERIMENTAL CRYPTORCHID TESTES AND THE RECOVERY OF SPERMATOGENETIC FUNCTION (GUINEA PIG).[1]

CARL R. MOORE.

HULL ZOÖLOGICAL LABORATORY,
THE UNIVERSITY OF CHICAGO.

The experimental work of Griffiths on the dog in 1893 proved that the testis of a normal breeding mammal, replaced in the abdominal cavity by operation, was converted within a few months into a degenerate testis devoid of an active germinal epithelium; the structure of such a testis was very similar to that found in testes congenitally retained in the abdomen. This structural condition, differing from the general opinion held until recently, is not the result of a faulty embryology but the result of an abnormal environment; the structural counterpart of the congenital abdominal testis can be duplicated in a very short time by moving the adult normal testis from the scrotum to the abdomen. Griffiths appreciated the fact that the assumption of this abnormal structural character was due to a change of environment, but he was entirely ignorant of the nature of the environmental difference, or the condition that contributed to the typical reaction.

In a restudy of this problem of experimental cryptorchidism (Moore, '24*a*) and many other aspects of the biology of the testis, we have obtained considerable information concerning the environmental influences and reactions within the mammalian testicle when this environment is modified. Since the literature on the biology of the testis and scrotum has recently been reviewed and discussed (Moore, '26*a*) only a few points of importance to the present study need here be mentioned (see also Moore, '26*b*, *c*, *d*). We have determined that a functional testis removed from the scrotum to the abdomen does not require months to show the destructive influences of the ab-

[1] This investigation has been aided by a grant from the committee on sex research of the National Research Council; grant administered by F. R. Lillie.

dominal position, but decided disorganization of the seminiferous tubules occurs within six days (guinea pig); the destructive effects are so pronounced and progressive that the testis is almost entirely cleared of its germinal epithelium within twenty to thirty days. As degeneration becomes more pronounced the testis as a whole diminishes markedly in size; the individual diameter of the seminiferous tubules is less; and usually the interstitial cells become decidedly more prominent. The testis does not again become active in spermatogenesis if it remains in the abdomen; two years after elevation it is but a small fraction of its former size, and entirely inactive in germ cell production. We have shown by many different lines of evidence that a higher temperature in the abdomen is the fundamental cause of its conversion into a degenerate testis and hence the scrotum has been recognized as a local thermoregulator which functions to produce a lower environmental temperature about the testis; it follows that such a regulation of temperature is necessary for the normal activity of the organ (Moore, '24*b*, Moore and Quick, '24).

It was pointed out (Moore, '24*a*) that replacement of a testis in the scrotum, shortly after it had been caused to degenerate, was followed by restoration of its gametogenetic function; figures representing the grade of degeneration at the end of twenty-four days abdominal retention and the recovery within a few months after scrotal replacement reveal the reëstablishment of spermatozoön formation. This emphasized the fundamental importance of the scrotal regulatory function on spermatogenetic activity.

The question has been raised many times since this publication whether a testis congenitally retained in the abdomen in man could later assume its normal function if it were replaced in the scrotum by an operation. And in attempting an answer to this important problem, further study has led to observations that form the basis of this report.

Material and Method.

The most usual cryptorchid condition encountered in nature (in man or other mammals) is a unilateral abdominal retention

with the opposite testis normal; obviously the testis at fault has never descended from the abdomen. In the guinea pig descent of the testis occurs before or immediately after birth, and in order to reproduce as nearly as possible congenitial abdominal retention, the animal must be operated very soon after birth. Guinea pig testes from five days to twenty days after birth (sometimes later) show but little tendency to differentiate into the elements of the germ cell line and usually spermatozoa are not found in the seminiferous tubules until from forty to sixty days of age. Operation can, therefore, be delayed until the animal has become quite self-supporting and the testis confined to the abdomen considerably in advance of tubular differentiation; with such procedures we are able, therefore, to reproduce essentially the conditions of congenitial retention.

The question of single or bilateral elevation of the young testis was definitely solved by our earlier experiments. It was pointed out that an abdominally confined, prepubertal testis reacted differently if its partner was normal or if it were either removed or likewise confined in the abdomen. When one testis is carrying on its normal gametogenetic activity a prepubertal testis in the abdomen remains essentially in an embryonic resting condition for months and even years; the seminiferous tubules retain essentially the same diameter, the cells do not perceptibly change their embryonic character, the interstitial spaces remain in fundamentally the same condition as at the time of elevation and the testis as a whole does not increase in size. If, however, both prepubertal testes of an animal are elevated to the abdomen, or if one is so elevated and the opposite testis removed, then the reactions are of a different character. The abdominal testis does usually increase in size, and the tubules in their diameter, but such an increase is essentially due to an edematous condition of the testis as a whole; the cells of the seminiferous tubules instead of retaining the embryonic condition become differentiated into cells believed to be Sertoli cells; the interstitial spaces are often much larger than the embryonic type and usually contain numerous and conspicuous cells of Leydig. This type of testis is not an embryonic type, but one that has undergone considerable modification. It is apparently very definitely

indicated that the experimental animal should have but one testis displaced into the abdomen while the other develops normally, and this report is restricted to material of this operative history.

The operative procedures, with perhaps minor variations, have consisted in opening the abdomen in a mid-ventral incision, retracting one testis into the abdomen, loosening its connections with the scrotal pouch, and fastening the testis high up in the abdomen by a suture passed through the epididymis and fastened to the body wall; this prevents possible return to the scrotal pouch, inasmuch as the inguinal canal was left open for future operative return of the testis to the scrotum. The opposite testis remained normal throughout the experiment or until the second, or replacement, operation at a later date. Four to five months after testis elevation the abdomen was opened a second time, the ligatures and adhesions cut and the testis replaced in the scrotal pouch in its usual position; a suture passing from the epididymis through the scrotal wall to the outside assisted in holding the organ in its desired location. Replacement of the testis without scrotal injury is not always successful. The absence of the growing testis from the scrotum results in a smaller pouch and coupled with this factor is the great increase in the fat body attached to the testis; often the testis to be replaced is but a fraction the size of its appended fat body. Attempts have been made to cut away the major portion of the fat body, without undue injury to the vascular plexus located within it, but the formation of adhesions of the entire mass replaced in the scrotal pouch have many times prevented a proper realization of the powers of the gland for recovery; adhesions often interfere decidedly with normal testicular function.

In most cases the opposite normal testis was removed at the replacement operation and on some occasions the degenerate testis was carried to the opposite side and replaced in the position of the normal testis in the larger scrotal pouch; the vas deferens often prevents the testis from reaching the bottom of the opposite scrotum and in some cases it has been purposely cut away to avoid such interference. It is now known that the testis does not have to be connected with its vas deferens to produce

spermatozoa. The testicular blood supply must of course remain undisturbed.

From three to five months after replacing the degenerate abdominal testis in the scrotum, the animals were killed and the testes so preserved and sectioned in paraffine as to make available entire cross sections of the organ from different regions. In some cases tests for motile sperm in the epididymis were made immediately after death. In every case autopsy revealed the replaced testis in a somewhat unfavorable condition for normal activity in as much as adhesions with the tunica vaginalis had formed; many times the testis with its fat body was firmly attached by adhesions to the bottom of the scrotum. Under such conditions the growth of the fat body undoubtedly brings excess pressure to bear upon the organ and in many cases the entire replaced testis was practically surrounded by fat. All such abnormal conditions minimize the chance of the testis being able to acquire its complete gametogenetic function. In a few cases adhesions were so formed that the position of the replaced testis was the equivalent of an inguinal canal retention or even lower abdominal retention; under all conditions, however, a portion of the testis came within the upper limits of the scrotum and was subjected to some extent to its influences.

Observations.

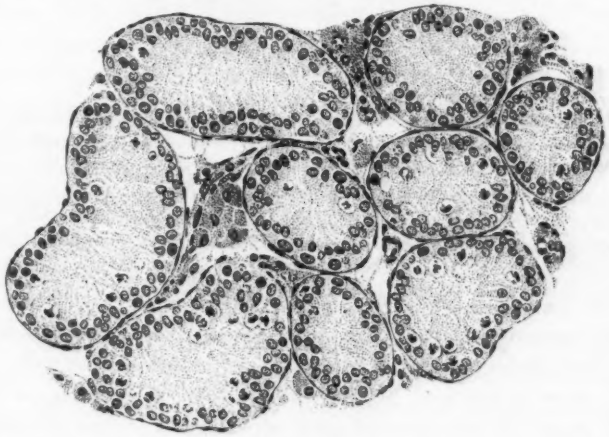
The observations here reported are based upon the most recent series of operations, involving a group of ten guinea pigs, in each of which one testis was removed from the scrotum to the abdomen at intervals from the 17th to the 42d day after birth involving no interference with the opposite testis (see Table I.). In nine animals the unilateral cryptorchid testis was replaced in the scrotum four or five months later; the tenth unilateral testis was removed at $4\frac{1}{2}$ months abdominal retention (animal No. 5) and prepared for histological study to serve as a control for the type of testis replaced in the scrotum of the other nine animals. In seven animals the opposite normal testis was removed during the operation for replacement of the cryptorchid testis and in the other two (Nos. 3 and 7) the normal testis remained intact throughout the experiment. The animals were

TABLE I.

No.	Animal Series.	Testis Elevation, Days after Birth.	Testis in Abdomen.	Testis in Scrotum.	Spermatozoa at Autopsy.	Remarks.
1	140(199)	17	4 months	4½ months	present	Normal testis to 2d oper.
2	141(200)	17	4 "	4½ "	present	" " " " "
3	142(201)	21	5 "	3 "	present	Normal testis throughout.
4	143(202)	21	5 "	3 "	present	Normal testis to 2d oper.
5	144*A* (211)	30	4½ "	—	—	Killed at 4½ months (Control) normal testis present.
6	144(208)	30	4 "	3 "	absent	Normal testis to 2d oper.
7	145(213)	30	4½ "	5 "	present	Normal testis throughout.
8	146(209)	30	4 "	6 "	present	Normal testis to 2d oper.
9	147(197)	42	4½ "	10 "	present	" " " " "
10	148(198)	42	4½ "	10 "	present	" " " " "

sacrificed at intervals from three to ten months after scrotal replacement of the abdominal testis.

In order to give a better understanding of the conditions of these testes before scrotal return, I reproduce here Fig. 1 (127*A*)



Practically only one tube

FIG. 1.[1] Seminiferous tubules of normal guinea pig testis seventeen days after birth (127*A*–218). Many mitotic figures but germinal epithelium shows little advance over embryonic condition.

[1] All drawings made by Kenji Toda.

to show the structural condition of the testis shortly after birth. It can be seen that this 17-day normal guinea pig testis consists of seminiferous tubules that show little or no tendency to produce an active germinal epithelium. The testis is essentially of the embryonic type and differentiation into the different elements of the germinal line does not occur until considerably later. When such a testis as this is confined to the abdomen it tends to remain in about the same stage of differentiation for several months and in fact after two years abdominal retention quite a similar picture is presented, provided the opposite testis is a normal one. If, however, both testes are confined to the abdomen, this characteristic embryonic type is not retained.

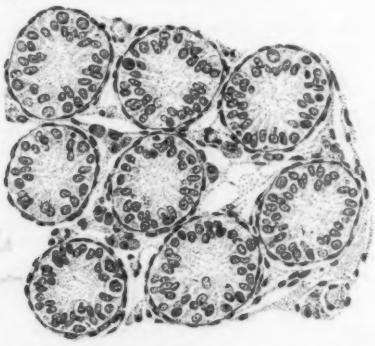


FIG. 2. Seminiferous tubules from a guinea pig testis (144*A*–211) elevated to the abdomen thirty days after birth (unilateral cryptorchid with opposite testis in scrotum) and removed 4½ months later. (Same magnification as Fig. 1.)

Fig. 2 (144*A*–211) is a reproduction of a few seminiferous tubules from the unilateral cryptorchid testis removed from animal No. 5 (see Table I.). It was elevated to the abdomen on the 30th day after birth and remained for 4½ months (opposite testis normal). Since this is approximately the average period of confinement in the abdomen for all cases reported here, it will serve to acquaint us with the grade of organization of all such testes at the time of their replacement in the scrotum. One can appreciate from Fig. 2 that although this testis was removed from the animal 5½ months after birth, its structural development has remained essentially that of the normal testis

of an animal seventeen days after birth; in other words the testis has shown practically no change from its condition at the time of elevation to the abdomen; it is prespermatic and has never produced a differentiated germinal epithelium (compare with Fig. 1) nor would it ever build such an epithelium were it allowed to remain in the abdomen. Returned to the scrotum, however, such a testis becomes active, builds a normal germinal epithelium, and produces motile spermatozoa.

In the nine animals (Table I.) in which the abdominally confined testis was replaced in the scrotum all but one (No. 6) subsequently produced spermatozoa. In two of the animals the testis was recovered after a period of but ninety days subsequent to replacement in the scrotum; two cases remained for $4\frac{1}{2}$ months, one for 5 months, one for six, and two for ten months. Development under the scrotal influences has, therefore, produced a testis differentiating spermatozoa within ninety days after its return from the abdomen.

The success in development varies with the success in scrotal replacement. In every replaced testis adhesions of variable extent had formed. This not only serves to modify even normal testis activity, but it has resulted in a variety of scrotal relationships. Some of the testes were firmly bound down to the bottom, or against the side of the scrotal pouch, and surrounded by the fat body normally attached to the anterior end of the testis; some were held firmly in the upper portion of the scrotum or in the inguinal canal; other testes were found to have been held firmly against the upper entrance to the scrotal pouch in a position more abdominal than scrotal, but in all at least one side of the testis projected sufficiently low to be palpable in the upper scrotum. Roughly paralleling the success of replacement in the scrotum is the success of the testis in developing normal spermatogenesis.

Let us take for detail consideration, animal No. 7 (Table I.). The right testis was removed from the scrotum into the abdomen on the 30th day after birth and allowed to remain high up in the abdomen for $4\frac{1}{2}$ months; the left testis was undisturbed. Since the operation periods were the same for this animal as that from which Fig. 2 was made, we can appreciate its structural

condition at the time of scrotal replacement. Having remained in the abdomen for 4½ months the testis from animal No. 7 was returned to the scrotum and allowed to remain therein for a period of five months. When the animal was killed this testis was firmly adherent to the bottom of the scrotum and more or less surrounded by its fat body. It cannot, therefore, be considered to have a normal environment on account of the con-

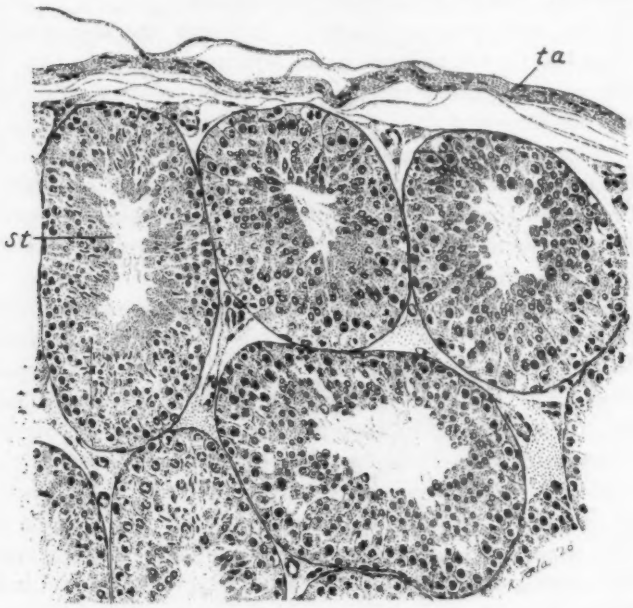


FIG. 3. Seminiferous tubules from guinea pig testis (145–213) elevated to abdomen thirty days after birth, remaining in abdomen 4½ months, replaced in scrotum for five months. Complete recovery in this area. *st*, seminiferous tubule with spermatozoa; *ta*, tunica albuginea.

stricting adhesions and the encapsulating fat. It was many times larger than when replaced in the scrotum and compared rather favorably in size with the normal testis opposite. From entire cross sections at three different levels one can see that the majority of the seminiferous tubules are in active spermatogenesis and a large percentage of these contain spermatozoa. Fig. 3 is a reproduction of a few normal seminiferous tubules,

some of which contain spermatozoa, and this figure should be carefully compared with Fig. 2 representing the structure of this testis at the time of scrotal replacement.

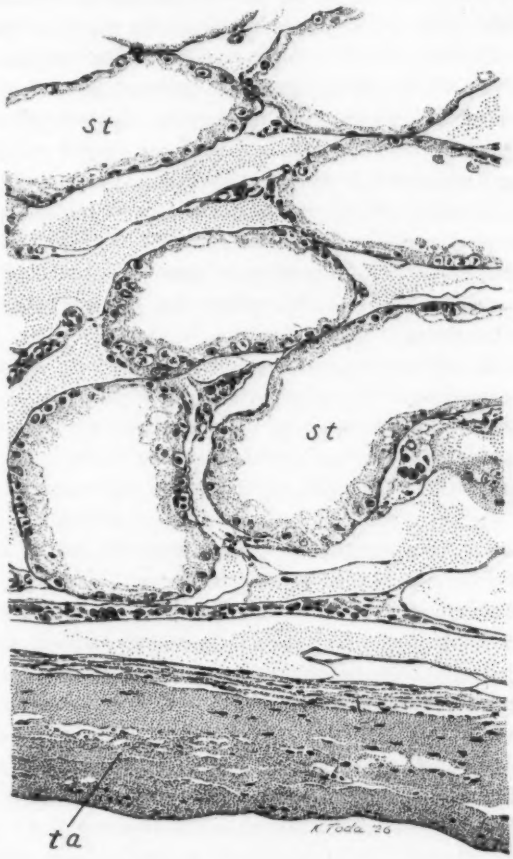


FIG. 4. Seminiferous tubules from same testis as Fig. 3 but from the side where adhesions were present. (Figs. 3, 4, 5 same magnification.) *st*, degenerate seminiferous tubules; *ta*, thickened tunica albuginea (adhesions).

Despite the establishment of normal seminiferous tubules throughout this testis since its replacement in the scrotum, there are many tubules that have not been restored to normal con-

ditions. Such tubules are more or less restricted to certain regions of the testis and may be entirely devoid of cells in mitosis or cells that appear to be members of the germinal line. Fig. 4 shows a small area from the same microscopic section as that from which Fig. 3 was taken, but from the opposite side of the organ which had formed adhesions with the surrounding tissues; this portion of the testis, therefore, was not located in a sufficiently favorable position for resumption of germ cell activity. Here the tubules have not, and probably never would have, developed an active germinal epithelium. One cannot always account for the localized regions of degenerate tubules but these are almost invariably found on the side of the testis where adhesions of the tunica albuginea to surrounding structures have been formed, or on the side where the fat body has closely surrounded the organ.

These degenerate areas within such a testicle do not interfere with the function of the normal areas. Spermatogenetic activity is continuous in the latter and spermatozoa are differentiated and transported to the epididymis; sections of the latter portion show the tubules filled with spermatozoa. Suspensions of these cells in 0.9 per cent. NaCl at the time of killing the animals revealed the spermatozoa to be as vigorously motile as those obtained from an entirely normal epididymis.

The difference between an abdominal testis and one replaced in the scrotum for a few months is indeed striking (compare Figs. 2 and 3). The entire development of the active germinal epithelium in the case cited, has followed replacement of the testis in the scrotum within five months. In animals 3 and 4 the replaced testis were found to contain quantities of spermatozoa three months after scrotal replacement despite the fact that the testis had been confined to the abdomen for a slightly longer period than the one considered in detail above. Development of the entire germinal line follows, therefore, within three months after scrotal replacement and appears as well established quantitatively as after a period of ten months in the scrotum (animals 9 and 10).

In the case of animal No. 7 the testis partner to the one replaced in the scrotum remained normal throughout the experiment but

it is not necessary that it should be present for development of the replaced testis. This is well shown in the experiment inasmuch as in animals 1, 2, 4, 8, 9, and 10 the normal testis was removed at the time of the second operation, and each of these six animals produced spermatozoa in the testis after replacement in the scrotum.

Out of the nine cases of scrotal replacement only the testis in animal No. 6 failed to produce spermatozoa. This testis was successfully replaced in the scrotal position, but numerous constricting adhesions had been formed and the testis recovered at the termination of its three months in the scrotum was considerably smaller than others residing in the scrotum for a similar period of time after replacement. Sections of this testis showed an entire lack of dividing cells in the seminiferous tubules; all cells present appear of the Sertoli type. It appears that all cells of the germinal line had been removed from the tubules and it is doubtful if this testis would have shown recovery effects if it had been allowed to remain in the animal for a considerably longer period.

The structure of the tubular portion of the testis in this case is typical of those cryptorchid testes that have not been opposed by a normal testis. Fig. 5 shows, in comparison with Fig. 2, that the tubules have undergone a change from the embryonic type to a type where apparently only Sertoli cells are present. The tubules are larger in diameter and lack undifferentiated cells, and the intertubular spaces are decidedly different from those of the undeveloped testis; interstitial cells of Leydig are prominent. Just why this testis should have failed to develop normal seminiferous tubules as did the other eight of the experiment similarly operated is not known unless it can be attributed to the constricting adhesions that developed about it after scrotal replacement.

The importance of the scrotum in controlling conditions that are essential for testicular activity can be shown by reference to conditions in which the testis was incompletely restored to the open scrotal pouch. In animal No. 1 scrotal replacement was incomplete inasmuch as the testis had developed adhesions and was held in a position essentially across the entrance into the

upper portion of the scrotum; in reality it was more nearly abdominal than scrotal. On recovery, however, the testis was very evidently much larger than at the time of the replacement

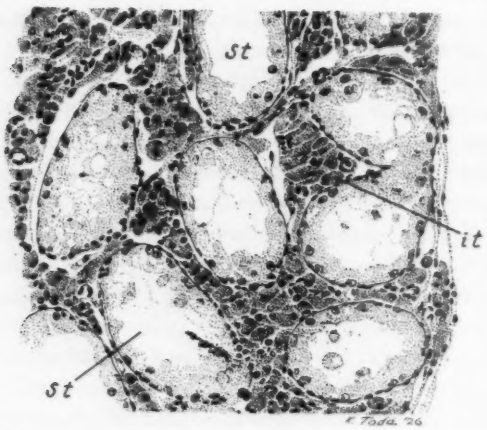


FIG. 5. Seminiferous tubules from guinea pig testis (144–208) elevated to abdomen thirty days after birth, remaining in abdomen four months, replaced in scrotum for three months (no recovery of function). *it*, interstitial tissue; *st*, degenerate tubules.

operation. Histological sections show that the majority of the seminiferous tubules were in active spermatogenesis, but very few of the tubules had developed to the stage of spermatozoön formation. Many of the tubules, though possessed of an active germinal epithelium, contained degenerating cells, multinuclear protoplasmic masses, and many vacuoles in the central portion of the epithelium. In other words, the testis was quite similar to testis grafts, heat applied testes, and subcutaneous testes in which spermatogenesis has continued but was usually never complete; the testis is not in an optimum environment for normal tubular activity. The few spermatozoön bearing tubules, however, show that the testis was in a position just on the effective borderline with a few tubules capable of completing spermatozoön formation.

Discussion.

A consideration of the observations here presented brings forcibly to our attention again the importance of the scrotum to the normal activity of the testes in those mammals for which a definite scrotum has been developed. It was pointed out earlier that testes caused to degenerate, after having once become active, by placing them in the abdomen for short periods of time would in some cases recover their normal function when replaced in the scrotum (Moore, '24*a*). Such a set of conditions, however, are obviously different from abdominal confinement before differentiation of the embryonic cells had taken place or from congenitally retained testes found in nature. And in order to gain an insight into the relative powers of undescended testes to acquire normal function after replacement in the scrotum, this series of experiments has been made; it may give us a relative answer to the possibilities of replacement of unilateral cryptorchid testes and recovery of function in man, but it is obvious that the answer is not absolute.

The conclusions are evident that a guinea pig testicle retained in the abdomen from shortly after birth until long after the establishment of sexual maturity, a condition essentially the same as a congenital retention of a testicle in man and a condition in which histologically similar testes are produced, can develop into a functional testis within ninety days after its replacement in the scrotum. Delay in the development of a testicle to its normal condition by abdominal confinement does not preclude its later development when it is brought into its proper environment. In regard to the application of such a conclusion from the guinea pig to essentially similar conditions found in nature, one must be very hesitant indeed in making more of the comparison than is justified. But if we may make so bold as to attempt a correlation between the findings here and their possible application to man, it would appear to be well indicated that we could expect scrotal replacement of a congenitally retained testis of a man long after the onset of sexual maturity, to produce a testis with normal spermatogenetic function within a relatively short period of time. In terms of years, it is more unsafe to predict the outcome. The guinea pig usually has produced

spermatozoa in its seminiferous tubules within sixty days after birth. By these operations we have prevented this and held the testis in an undifferentiated condition for a period longer than four months, yet have had recovery of function on scrotal replacement; the implications from testes confined longer in the abdomen are that similar results could have been obtained from testes confined in the abdomen for over a year. Since three years may fairly well represent the average duration of sexual function in the guinea pig, one who is interested may carry the implications to relative periods of retention in the human individual and perhaps gain some insight to possibilities existing in such conditions. From the fact that recovery in the guinea pig may follow many months after the onset of sexual maturity, we might expect that in the human individual of twenty-five to thirty years, and from implications one considerably older, a scrotal replacement might result in the production of a normal testicle. It is obvious that these implications, though basically sound, require exact study before they can be more than implications. From the nature of the case it is obvious that since relatively few operations of this character are now attempted on the human individual, and since it is not frequent that such a replaced testicle will come to histological study, there is but little exact data in the literature of any significance in making such comparisons. Bevan ('18), among other surgeons, has given considerable study to the problems on the human individual, but it is well recognized that with one normal testis besides a scrotally replaced one, about the only criterion of effectiveness in its replacement would have to come from size and firmness determined by palpation or perhaps from hypodermic syringe samples.

The nature of the influence on a prepubertal abdominally retained testis by the opposite normal testis is not clear; the spermatogenetic activity, however, does exert some influence on the character of the undescended testis. Lipschutz ('25) has recently shown that the expression of the ovarian influence in a male animal by an ovarian transplant is likewise conditioned by spermatogenetic activity; a normal testis prevents the expression of the hormone influences of a transplanted ovary whereas a

cryptorchid testis does not prevent it. The effect of the influence on a unilaterial abdominal testis is expressed by an almost entire suppression of any developmental capacities. In contrast to this the cryptorchid testis uninfluenced by spermatogenesis in another testis undergoes decided modification. In earlier reports (Moore, '24*a*) it was noted that replacement of abdominal testes that had undergone this change was not followed by normal gametogenetic function. It is believed that the degeneration of such testes had proceeded to the extent that all germinal cells were removed from the tubules.

The radical difference between the four or five month cryptorchid testis here described and the condition following within 90 days after replacement in the scrotum, serves as a striking demonstration of the difference between the capabilities of a given testis retained in the abdomen and one replaced in the scrotum.' Having remained embryonic in character and relatively quiescent for months, it rapidly becomes engaged on a constructive program and fulfills the primary function of the organ before the end of three months. Thus the scrotal influence is necessary for the expression of the potentialities of the organ and the reader is referred to previous articles for the many lines of evidence that have led to the conception that the effective principle involved is a heat regulatory function on the part of the scrotum.

Summary and Conclusion.

1. Guinea pig testes elevated from the scrotum to the abdomen shortly after birth (prespermatogenic) will, if opposed by a normal testis, retain an undifferentiated character practically throughout the life of the animal. If unaccompanied by a normal testis, they undergo changes leading to the enlargement of seminiferous tubules lined with cells of Sertoli, relative increase of interstitial cells, and a general condition unlike that of the embryonic type.

2. Replacement in the scrotum of the testis retained in the abdomen for five months was followed by normal activity and differentiation of spermatozoa in eight out of nine cases. Presumably normal development of abdominal testes confined in the abdomen for much longer periods would follow scrotal replacement.

3. Spermatozoa are produced in the testis within ninety days after scrotal replacement.

4. Development of the replaced testis occurs in the presence or absence of an opposite normal testis.

5. By implication we are led to believe that congenitally retained abdominal testes in man have a good chance of developing into normal testes if they are replaced in the scrotum at a time considerably after the attainment of sexual maturity.

6. The function of the scrotum is beautifully shown by comparison of the testis retained in the abdomen for several months with a similar testis replaced in the scrotum for ninety days. Only in the scrotum are spermatozoa produced in the testes.

LITERATURE CITED.

Bevan, A. D.

'18 Undescended Testes. Surgical Clinic of Chicago, 2: 1101–1117.

Griffiths, Joseph.

'93 The Structural Changes in the Testicle of the Dog when it is Replaced within the Abdominal Cavity. Jour. Anat. and Physiol. (Lond.), 27: 482–499.

'94 Retained Testes in Man and in the Dog. Jour. Anat. and Physiol. (Lond.), 28:209–220.

Lipschutz, A., and others.

'25 Experimenteller Hermaphroditismus und der antagonismus der geschlechtsdrusen. I–III. Pflugers Archiv, 207: 548–595; IV, V, *ibid.*, 208: 273–317.

Moore, Carl R.

'24*a* VI. Testicular Reactions in Experimental Cryptorchidism.

'24*b* VIII. Heat Application and Testicular Degeneration; the Function of the Scrotum. Amer. Jour. Anat., 34: 337–358.

'26*a* The Biology of the Mammalian Testis and Scrotum. Quart. Rev. of Biol., 1: 4–50.

'26*b* IV. Testis Graft Reactions in Different Environments (Rat). Amer. Jour. Anat., Vol. 37: 351–416.

'26*c* The Activity of Displaced Testes and its Bearing on the Problem of the Function of the Scrotum. Amer. Jour. Physiol., Vol. 77: 59–68.

'26*d* The Relation of the Scrotum to Germ Cell Differentiation in Gonad Grafts in the Guinea Pig. Amer. Nat., Vol. (In press).

Moore, Carl R., and Quick, Wm. J.

'24 The Scrotum as a Temperature Regulator for the Testes. Amer. Jour. Physiol., 68: 70–79.

THE FATE OF THE GERMINAL EPITHELIUM OF EXPERIMENTAL CRYPTORCHID TESTES OF GUINEA PIGS.[1]

WALTER LAWRENCE,

HULL ZOÖLOGICAL LABORATORY,
THE UNIVERSITY OF CHICAGO.

The experimental work of Moore and his students on the biology of the testis has yielded many interesting results and has pointed the way to some further analyses of the activities of this organ. Moore ('24*b*) has discussed at some length the reactions in a normal breeding guinea pig testis that has been removed from the scrotum and confined in the abdomen. The germinal epithelium rapidly becomes disorganized, undergoes degeneration, and the contents of the seminiferous tubules are almost completely removed within a very short time. Such a rapid degeneration is not followed by recovery of the generative portion as long as the testis remains in the abdomen; return to the scrotum, however, was followed by recovery to the extent that the testis again produced spermatozoa. This and many other determined facts emphasized the relation between a scrotal position and the germinal content of the organ and has led to the development of the idea that the scrotum is a local temperature regulator for the testes and that such a regulation is necessary for the production of spermatozoa (Moore, '24*c*; Moore and Quick, '24*a*).

Despite our general knowledge of the progressive degeneration of a testis placed within the abdomen we yet have much of importance to determine in the ultimate fate of the cells of the germinal line. Are the contents of the epididymis affected in the same manner and as rapidly as those of the seminiferous tubules? How long can spermatozoa be found in the genital passages of cryptorchid testes, and what is the relationship

[1] This investigation has been aided by a grant from the committee on sex research of the National Research Council; grant administered by F. R. Lillie.

between morphology and motility of spermatozoa? These and other questions are discussed in the following pages of this paper.

It was at the suggestion of Professor Carl R. Moore that I attempted this phase of the sex problem, and I wish here to express my appreciation for this and for his kindly direction during the progress of the work.

Observations.

For the purpose of studying the progressive changes in the degenerating testis thirty-three guinea pigs have been used in which by operation one or both testes have been confined in the abdomen for relatively closely graded periods of from six to one hundred and thirteen days. The operation is simple and consists of opening the abdomen, freeing the testis attachment to the scrotum, and elevating the testis through the open inguinal canal into the abdomen; to secure permanency in this position the inguinal canals have been closed by sutures. The blood supply, nerve connections and ductus deferens are thus subjected to no interference.

A. Seminiferous Tubule Degeneration.

Six-day Cryptorchid Testes.—Observed at the end of six days retention in the abdomen the testis of an adult breeding guinea pig shows but minor macroscopic changes; the organ may appear somewhat hyperæmic. Microscopical changes, however, are pronounced. The majority of the seminiferous tubules are abnormal, although the amount of alteration is variable. The diameters of the tubules are unchanged, and the intertubular spaces appear normal. In most cases the contents of the tubules are so shattered and disarranged as to bear little resemblance to the normal condition, while in a few the zones of spermatogonia, spermatocytes, spermatids and spermatozoa can be made out. On more careful examination individual cells within the tubules are seen to be swollen and granular, and in many cases more or less completely fragmented; numerous vacuoles appear among the cells constituting the germinal epithelium. From this it can be readily determined that some rapidly destructive process has involved all the tubules. The greater part of the

lumen in most cases is filled with a mass of cells and debris, obviously in a state of parenchymatous degeneration. The intact cells are swollen and granular, and edges varying from perfectly smooth to a very irregular outline, and the nuclei likewise lose their identity; some of the latter appear eccentric, others fragmented, while in some of the degenerate masses no evidence of nuclei are to be seen. Of the degenerating cells in this central mass spermatids are most abundant, although many spermatogonia and spermatozoa may be seen. The latter, though least altered morphologically are most atypical in their position. Tufts of spermatozoa which normally border on or near the lumen are sometimes seen with their head almost touching the basement membrane of the tubule. Some of these masses are straight while others are adherent to the periphery of spheroidal masses of granular material. The other cellular elements have a tendency to coalesce and groups resembling typical giant cells of the myeloid type may be seen. The most common characteristic throughout all the tubules is the presence of large clear vacuoles in the germinal epithelium. These are most abundant near the periphery of the tubules, although they may be present in any portion of the cross section. Within some of these vacuoles fragments of more or less completely autolyzed cells are seen. There is little doubt that these vacuolar cavities represent areas of degeneration and liquefaction of epithelial cells.

We see therefore that a normal adult guinea pig testis removed from the scrotum to the abdominal cavity is decidedly degenerate at the end of six days. Six days after operation is the earliest stage studied, but it is apparent that the effects could have been noted at least one, or possibly two days earlier. In a given cross section of the entire testis, approximately 95 per cent. of the tubules are atypical, the remainder approximating the normal. In the most pronounced degeneration the germinal epithelium is so radically affected that it is in complete disorganization, and the lumen of the tubule may be all but obliterated by whole and fragmented cells from the epithelium.

Ten-day Cryptorchid Testes.—A testis retained in the abdomen for ten days is slightly smaller than the normal; this is clearly

demonstrated where one testis is allowed to remain in the scrotum while its fellow is elevated to the abdominal cavity. In this case the cryptorchid testis is about four fifths the size of the normal.

The beginning degeneration noted in testes of six-day abdominal retention is now greatly advanced. The vacuoles which characterized the preceding stage have become more or less confluent, and in many tubules the desquamated epithelium is not so abundant, so that the lumen of the tubules is now larger and more open than in the previous stage. The lumen is not completely reëstablished in all cases; in many of them the central area is still occupied by wide vacuoles which have not as yet coalesced, while a few tubules are still cord-like in appearance. Cells showing advanced necrosis are present in the lumina of the seminiferous tubules and most of these are discrete, but some are agglutinated into masses which appear as a single large protoplasmic mass with multiple nuclei, the so-called giant cells. Of these degenerating elements within the lumen, many can be identified as spermatocytes and spermatids, but not as spermatozoa. Around the periphery, however, many spermatogonia and spermatocytes still remain, while an occasional spermatozoön may be seen not far from the basement membrane.

The blood vessels are somewhat more dilated in this testis than in the normal and an extravasation of serous exudate into the intertubular spaces, and a diapedesis of leucocytes from the capillaries may be seen.

A testis retained in the abdomen for ten days is, therefore, considerably more degenerate than one retained for six days, the chief difference being that in the ten-day retained testis there has been further degeneration of the cellular elements with a widening and often a confluence of the vacuoles.

Sixteen-day Cryptorchid Testes.—The organ appears about two thirds the size of an unoperated testis, and presents no superficial evidences of congestion. Microscopically the tubules show a decrease in diameter, as well as a diminution in the germinal epithelium content. The lumen in most cases is narrowed or even filled in completely by a thin reticulum of Sertoli cell cytoplasm. The number of Sertoli cells is apparently increased,

due in all probability to the smaller caliber of the tubules, and a concentration of these cells, and they appear here as a simple epithelial lining for the tubules. With the exception of a few spermatogonia situated near the periphery of the tubules, intact germinal epithelium is practically absent. A few cells of degenerating germinal epithelium are seen free in the lumen; the rete tubules also contain some degenerating material that is being transported to the epididymis as will be pointed out below. The intertubular spaces show slight evidences of vascular stasis; the interstitial cells show no obvious change. The tunica vaginalis is somewhat thickened.

Thus by the end of the sixteenth day of abdominal retention, practically all of the former conspicuous germinal epithelium has been removed from seminiferous tubules, leaving chiefly a lining of Sertoli cells.

Twenty-day Cryptorchid Testes.—The testis confined to the abdomen for twenty days is smaller than the preceding organ, and measures slightly more than half the diameter of the unoperated testis in the same animal. The germinal epithelium is still more reduced here, so that only an occasional spermatogonium may be identified. In about half of the tubules the lumen is distinctly widened at the expense of the Sertoli cell cytoplasm, so that the lumen occupies from one half to two thirds the diameter of the tubule. The interstitial cells are slightly more conspicuous here and the intertubular spaces contain considerable serous exudate.

We see therefore that by the end of the twentieth day the process of evacuation of the germinal elements is complete, with the exception of a few spermatogonia, which retain their normal position between the bases of the Sertoli cells.

Abdominal Retention for Longer Periods.—Despite the fact that my interest has been primarily in the direction of the disposition of the degenerating epithelium, and that by the end of the twentieth day of abdominal retention practically all of this has been transported from the body of the testis, it has been found necessary to study the epididymis after longer retention. A word or two in reference to the seminiferous tubules of these testes, retained for from twenty-five to one hundred thirteen days, will not be out of order.

Moore ('24*b*) has already shown that a normal breeding guinea pig testis artificially retained in the abdomen loses practically all its germinal epithelium within twenty days and that it does not recover from this striking degeneration as long as it remains in the abdomen. My own observations confirm Moore's assertions that degeneration of the seminiferous tubules is marked after six days, that the degenerative process is progressive, and that by the twentieth day the tubules contain only the Sertoli syncytium within which are a few spermatogonia.

In my series of experiments testes retained from 25–113 days in the abdomen show no signs of seminiferous tubule recovery; indeed degeneration is more pronounced in that the testis becomes progressively smaller so that by the end of the second month the whole testis is but half the diameter of the opposite or normal organ. In these testes retained for two months or more in the abdominal cavity the diameter of the seminiferous tubules is greatly reduced and the interstitial cells occupy the greater part of the intertubular spaces; there is also more or less serous exudate between the tubules. The tunica albuginea is five or six times as thick as that of the normal testis.

It is apparent therefore that active degeneration of the germinal epithelium begins some time before the sixth day, is most rapid from the sixth to the tenth, during which time the greater part of the germinal epithelium is shed; succeeding changes have to do chiefly with the Sertoli cells which seem to be increased, the diameter of the tubules, and the relative abundance of interstitial cells. The rapidity of the transformation seems to be quite constant for all animals studied.

Vas Deferens Ligation of Abdominal Testes.—Seminiferous tubule degeneration, as pointed out above, is of a varied character; some cells are thrown out into the lumen, some apparently undergo solution in situ, the autolyzed products being absorbed directly into the blood stream. The question arose in reference to this degeneration, whether a part of the fragmenting cells and general debris in the lumen of the tubules were carried into the epididymis and eliminated from the body through the genital passages, or whether it was wholly or partially absorbed into the blood stream from the testis. To demonstrate just what

elements are eliminated through the vas a number of animals were used in which both testes were elevated to the abdomen, and the vas deferens of one ligated in order to make certain the confinement of all testicular material to the organ in question. The degeneration of this vas-ligated, abdominally-retained testis was compared in its progressive stages with a similarly confined though unligated testis of the opposite side, and with testes with similar periods of retention in other animals. Macroscopically the two testes, one with ligated vas and the other with non-ligated vas when compared after any length of retention were entirely similar; one is unable to note difference in size or in vascularity. Microscopically also the two are similar. Degeneration begins as early in the testis with non-ligated vas as in the one with ligated vas, and the tubules of each are cleared of germinal epithelium in approximately the same time. So far as the seminiferous tubule degeneration is concerned, therefore, ligation of the vas deferens of an abdominally retained testis is without effect. The significance of this added ligation of the vas is demonstrated in the following section where the contents of the epididymis are considered. I had found that some of the degenerating cells, and some cells apparently not so degenerate were transported to the epididymis. Are any of these cells eliminated from the testis through the vas deferens, or do they go into solution in the epididymis and pass out entirely in the blood stream? Comparison of epididymal contents in testes of equal retention where one vas is open and one closed should give us an answer to this problem.

B. The Degenerative Process in Relation to the Contents of the Epididymis.

The epididymis forms a cap-like extension on the posterior pole of the testis, and is somewhat conical in contour, with the base adjacent to the body of the testis. Examined macroscopically in a normal pig it is seen to consist of tubules of varying size, largest at the apex and smallest at the base, and covered with a transparent membrane, the tunica vaginalis, through which the tubules appear as small while cords. The larger tubules at the apex constitute the ampullæ of the ductuli

efferentes, which become smaller toward the base of the epididymis. These tubules in a sexually mature male are normally distended with spermatozoa which are suspended in a viscous matrix. On opening one of these normal tubules the contents exudes as a thin white paste-like substance which on microscopic examination reveals innumerable spermatozoa; these cells are usually immotile until a drop of physiological saline solution is added, when they are activated to rapid motility. Anderson ('22) in a series of experiments on the spermatozoa of various domesticated animals finds that sperm are normally immotile until they are activated by the accessory secretions of the reproductive system, but that the same results may be obtained by adding normal saline. Armstead ('25) working with guinea pigs finds sperm are made motile by addition of exudate from the seminal vesicles which action he attributes to the alkalinity of the exudate. I have observed slight motility in freshly expressed spermatozoa, even before the saline was added but this was a weak vibratile motion. When the saline is added the spermatozoa became very motile, and are seen to swim about in the medium in groups of from two to eight or ten, the heads being cupped together, not unlike the condition seen in blood where the erythrocytes have a tendency to form rouleaux. In this stacked condition the flagella point in the same direction, but there appears to be no rhythm in the motion of the flagella, some vibrating vigorously and some little or none at all; as a consequence the locomotion of these sperm groups is slow and cumbersome, particularly so in the larger groups.

In a cross section of the normal epididymis of the guinea pig the tubules are all seen to contain a greater or less number of spermatozoa with a marked tendency for agglutination as already described. The ductus epididymis contains a mass of sperm usually centrally located; the ductuli efferentes are more completely filled and the ampullæ of these tubules are thin walled sacs well distended with sperm. The ductus deferens, likewise contains a large number of sperm throughout its course.

Epididymis of Six Days Abdominal Retention.—Macroscopically the epididymis of a six-day abdominally retained testis shows slight differences in size and color from the normal. It is slightly

smaller and its base has a somewhat glassy appearance, whereas the normal epididymis is milky white throughout. The contents as seen in cross section of the distal portion are very similar to those of the normal epididymis, and are distinguished by a few more round cells than are to be found in the normal. Toward the base of the epididymis the number of round epithelial cells is seen to increase, and along with these a considerable amount of degenerating debris all intimately mixed with varying amounts of spermatozoa. These germinal epithelial cells, in various stages of degeneration, have been carried over from the testis as a result of the liberation of these elements from the germinal epithelium through abdominal retention. Spermatozoa in the distal portion of the ductus epididymis and the vas deferens were similar to those of normal testes.

As it is important to know the effect of abdominal retention on the spermatozoa contained in the epididymis at the time of its removal from the scrotum, stained smears from the epididymis and vas deferens have been prepared in all cases after abdominal retention. Physiological saline has also been added to freshly expressed material from these localities in order to determine the duration of motility of spermatozoa. Mallory's triple stain has proven especially valuable here, as the head caps stain blue while the rest of the sperm body is stained red. Smears from the epididymis of a six-day abdominal testis show the sperm to be morphologically normal, and occurring in clusters as described above. On addition of saline the spermatozoa were seen to be as motile as the normal, moving about in the characteristic clusters, showing that as far as one can detect, the spermatozoa contained within the epididymis are in no way effected by the abdominal retention of six days.

Epididymis of Eight Days Retention.—A testis retained in the abdomen for eight days shows the base of the epididymis slightly glassy in appearance. In section the distal portion of the ductus epididymis was seen to contain an abundance of sperm, while in the proximal portion of this tubule degenerating elements predominate. For the most part the ductuli efferentes contain no cellular elements, being filled with a homogeneous liquid which stains faintly. In certain areas of the epididymis, and

especially between the coils of the ductus epididymis are many polymorphonuclear leucocytes and indeed these are within the tubule in some areas. Ligation of the ductus deferens at the time of testis elevation produces no outstanding differences in the epididymal contents.

Sperm expressed from the ductus epididymis and ductus deferens as well as sperm observed in the prepared material are seen to be morphologically intact, but they have lost some of their tendency toward agglutination. When physiological saline was added to these spermatozoa, only a slight amount of motility was observed and this was a weak vibratile motion. No difference in the morphological or physiological characteristics in the sperm from the epididymis and vas could be discerned, nor was there any difference between the sperm from the ligated and non-ligated sides. It should be remembered, as pointed out above, that the seminiferous tubules of the testes are at this time at the height of degeneration with the lumina packed with degenerating remains, and that but few sperm could be found among these; transfer has continued from the seminiferous tubules since the sixth day, many of the degenerating cells being present in smears and sections of the epididymis. The sperm seen here are undoubtedly at least eight days old, and their viability is a point not to be overlooked.

Epididymis of Ten Days Retention.—The gross appearance of these epididymi is similar to that seen in the preceding stage. Where the vas is ligated the epididymis is somewhat larger and the basal portion shows a clear zone. At the end of ten days the ductus epididymis is fairly well distended with sperm, very little diluted with other constituents; this is true of both the ligated and unligated sides. In the proximal portion of these tubules fewer sperm are present with an abundance of spherical epithelial cells from the seminiferous tubules, and are characterized by their tendency to form spherical masses. These cells are identical with those described as giant cells in the degenerating seminiferous tubules. Some of the proximal tubules contain deep staining particules of irregular shape and dimensions immersed in a homogeneous coagulum. Sections show that in the proximal tubules in these epididymi the sperm have lost all

tendency toward clumping, but in the distal portion this tendency is still present; however, smears made from this stage show no tendency toward clumping, and no sperm motility could be observed when physiological saline was added. In neither the epididymis nor the vas did the sperm show any obvious morphological change. It seems apparent therefore that as a criterion by which to judge of viability the tendency toward clumping is a better index than the morphology of the individual sperm.

The Epididymis of Sixteen Days Retention.—The epididymis retained for sixteen days in the abdominal cavity shows a glassy appearing zone three or four millimeters wide at the base. The entire organ is smaller than the normal, whether the vas is ligated or not, and where not ligated it is smaller and presents a broader clear zone at the base than in the ligated one. In sections the distal tubules are seen to contain spermatozoa which appear normal except for the clumping tendency. Further toward the base of the epididymis the number of spermatozoa becomes less, and at the extreme base there are few or none; instead there is to be seen a coagulum of fragmenting elements, degenerate beyond identification. Between these extremes some of the tubules contain a few spermatozoa mixed with a large number of swollen epithelial cells of varying size, among which are characteristic globular masses of these epithelial constituents. Polymorphonuclear leucocytes are common within and between the tubules in the basal portion of the epididymis.

In comparing the epididymis of the ligated and non-ligated vas, we find the latter somewhat smaller, the number of sperm less, and the amount of degenerating debris relatively more. This is easily explained since the ligature prevents the outward flow of sperm in the epididymis and vas, so that the degenerating constituents entering from the testis are able to occupy only the proximal tubules. The normal fellow of a cryptorchid epididymis is normal both as to appearance and contents and is apparently affected not at all by abdominal retention of the opposite testis.

The smears of the vas and epididymal contents from the testes of sixteen-day retention show sperm which are to all appearances normal morphologically, but which exhibit no pronounced tendency to clump, and are not activated to motility by the addition of physiological saline.

The Epididymis of Twenty-day Retention.—There is little difference between this and the preceding; the tubules proximal to the testis contain either degenerating debris, or sperm highly diluted with epithelial cells all of which are products recently evacuated from the testis. The distal tubules contain chiefly spermatozoa, and although most of these are normal, some show separate head caps and stain slightly fainter than freshly killed spermatozoa. It is a noteworthy fact that although the germinal epithelium of the seminiferous tubules is markedly disarranged and shows considerable degeneration at the end of six days abdominal retention, the mature spermatozoa present in the epididymis at the time the organ was elevated to the abdominal cavity show noticeable changes only after twenty days, a time when the seminiferous tubules are almost completely denuded of their germinal epithelia.

Twenty-five to One Hundred and Thirteen Days Retention.—With longer abdominal retention the vitreous area at the epididymis is increased, so that by the end of the second month the whole epididymis has lost its milky appearance. This difference in appearance is brought out by the fact that the liquefied and degenerating materials from the testis proper have mixed with the spermatozoa at the base and the latter have likewise slowly yielded to the degenerative process so that ultimately the entire organ contains only liquefied and fragmenting material which presents a vitreous appearance. That this material remains in the epididymis to be absorbed is shown by the fact that at the end of 113 days abdominal retention the tubules still contain a small amount of degenerating debris whether the vas is ligated or not. It should be mentioned here that the process of degeneration and absorption are much slower in the ductus deferens since sperm retained here proximal to the ligature are still identifiable after 113 days retention, whereas those retained in the efferent ducts are completely degenerated.

It is reasonable to suppose that with the addition of the degenerating products from the seminiferous tubules to the already distended epididymis that the latter would be distended beyond its normal size where the vas was ligated, or that an overflow would occur with the vas unligated. Observation,

however, shows this to be true only in the early stages of degeneration, for in all cases of abdominal retention for longer than sixteen days the epididymis is actually smaller than the normal. This may be accounted for by the supposition that absorption rapidly removes the autolyzed portion of the degenerating material, and at the same time concentrates the contents already present in the tubules. On cutting one of these tubules and expressing some of the contents it is seen to be of much thicker consistency than that of the normal tubule.

Sections through epididymi at various levels and with varying periods of abdominal retention bear proof that the spermatozoa degenerate very slowly; this process, however, varies somewhat in the same organ, being slower in the thicker walled portion of the ductus epididymis and more rapid in the efferent ducts. In the vas sperm retained their integrity for 113 days, while in the ampullæ of the ductuli efferentes degeneration was apparent after 20 days. In the process of degeneration the spermatozoa first lose their head caps at which time they stain poorly, later the tail separates and the body undergoes granular degeneration.

Spermatozoa in Other Passages.—As a part of the routine examination of the urogenital passages of the animal at autopsy, the seminal vesicles and urinary bladder were examined by making fresh smears for the presence of spermatozoa. This was done by aspirating some of the contents of the urinary bladder with a hypodermic syringe and by opening the seminal vesicles at various levels and making fresh mounts of the contents. In no case were sperm found in the urinary bladder. In five cases sperm were recorded from a seminal vesicle; in two cases a single sperm was seen in each; in two cases the number was described as "few;" and in one case they were abundant. This observation was made before it was realized how very easy it is to carry over sperm from a severed vas on scissors or instruments. My observations, therefore, confirm the findings of Fisher ('23) and Armstead ('25) who demonstrated that the seminal vesicles in guinea pigs are not sperm reservoirs. In two cases, when the animals were killed by a blow on the head there appeared on the phallus a mass of material the size of a pea, which to the feel was very rubbery, and which upon exam-

ination was found to contain many motile spermatozoa, mixed with exudate from the seminal vesicles.

As a result of this series of observations it becomes apparent that the degenerating products of the seminiferous tubules due to abdominal retention are removed chiefly by way of the blood stream. My study leaves little doubt that a great deal of cytolysis goes on in the early stages of degeneration in the seminiferous tubules, and it is highly probable that these materials are removed from the testis by the circulation; but it is also apparent that a great deal of the degenerating material is transported to the epididymis. Since the tubules of the testis are cleared by the 20th day, and many times earlier, and since the epididymis, even with its vas normally open, contains much of its normal sperm content it is clear that the degenerating cells are not removed from the epididymis through the vas in any considerable amount. Indeed it is not apparent that the majority of the spermatozoa contained within the epididymis are so removed. My material leads me to believe that practically all the degenerating products from the seminiferous tubules are liquefied and absorbed in the blood stream, either from the testis proper, or the epididymis, and that a large part of the spermatozoa in the epididymis are likewise liquefied and absorbed.

C. The Vitality of Spermatozoa.

The question of the vitality of spermatozoa has been long debated, and extensive investigations have been made to determine just how long spermatozoa will live in the uterus; less attention, however, has been paid to the life of the sperm in the male genital passages. De Lee ('21, p. 21) states that human spermatozoa will live for 21 days in the human uterus. Nurnburger ('20) claims to have kept human spermatozoa alive in the laboratory for seven or eight days. Hoehne and Behne ('14) claim to have demonstrated that all sperm die in the human vagina in one and a quarter hours, and that in the uterus no live sperm may be found after three days. Lewis ('11) bred 20 sows to boars which were known to be fertile, after which the sows were slaughtered and the uteri examined. He states: "In only two cases were sperm found alive 24 hours after

breeding." Anderson ('22) has pointed out that sperm are very sensitive to chemical and temperature changes. He was also able to find a few live sperm in the uterus of a mare seven and one fourth hours after breeding; and in hens sperm were found six to eight hours after breeding, but after fifteen hours none could be found. Wolf ('21) was able to keep rabbit sperm alive for nine days by the use of properly balanced physiological saline and isotonic glucose solution, and the required P_h concentration. Many more such references might be cited, but this will serve to show something of the diversity of the results obtained by different workers. Although there is doubtless a great variation in the viability of sperm from different species, doubtless some difference in results are attributable to the environment to which the sperm was subjected.

In this series of experiments particular attention was paid to the vitality of spermatozoa. When an animal was killed the vasa and epididymi were examined for sperm and where found were tried for notility by adding physiological saline to the contents. As stated above spermatozoa were found alive in the epididymis as long as eight days after both testes were elevated to the abdominal cavity, and closely associated with this there was a strong tendency toward dissociation of the clumps so characteristic of viable guinea pig sperm. To recheck on this very important point five pigs were used. Both testes were elevated to the abdomen, both vasa ligated, the left with a single ligature near the posterior pole, and the right in two places, one near the epididymis and the other one inch below, so that on the right side, the sperm which were in the lumen at the time of operation were not allowed to escape, and no more sperm could enter. When the animals were killed the contents of the epididymi, and of the vasa above, between and below the ligatures were examined and in each case the results were the same. The animals were killed on the 7, 9, 11, 13 and 14th days after operation. In only the first two cases, that is after seven and nine days were motile sperm found, and in these cases no difference in motility could be detected whether the sperm were taken from the region above, between or below the ligatures. In both these cases the motility was accompanied by the clumping

phenomenon described in a preceding paragraph. Although there was motility in some of the sperm after nine days abdominal retention the per cent. which were motile was considerably less than in the case just cited. With the loss in motility the tendency to clump is lost, so that in sperm from a ten-day abdominally retained epididymis there is little or no clumping, although the sperm bodies may appear approximately normal for a month longer in the ductus epididymis and vas.

It is a surprising fact in view of the extremely rapid degeneration within the seminiferous tubule that spermatozoa morphologically normal are found in the epididymis for such long periods of time after all other elements have disappeared. Thus within twenty days the seminiferous tubules are approximately empty whereas the epididymis contains sperm apparently normal so far as morphology is concerned for thirty-seven days. It is also significant that the rapidity of degeneration of spermatozoa varies in different parts of the passages. Thus in the efferent ducts and in the proximal portion of the ductus epididymis degeneration is marked at twenty to thirty days, in the thicker walled distal portion of the ductus epididymis and vas sperm are considerably less altered after retention of 113 days. To account for this difference in rapidity of degeneration is beyond the scope of this paper, but it might be suggested that probably the cause is referable to the difference in vascularity of these parts.

Since in the vas, degeneration is least marked, and since this is the real reservoir for mature sperm, it would be reasonable to suppose that vitality would be retained as long or even longer than any place inside or outside the animal body, as here they are surrounded by what would be supposed to be the most suitable environment. As sperm are capable of showing motility for nine days in this location after ligature, and as these sperm were present an indeterminate length of time before operation, we can state with assurance that the maximum life of the guinea pig sperm in the male genital tract is more than nine days.

D. Vascular Changes in Relation to the Degenerative Process.

Having noticed that testes made cryptorchid for from six to sixteen days were somewhat hyperemic as evidenced by the

dilated condition of the superficial veins and the presence of serous exudate in the intertubular spaces, it occurred to me that perhaps degeneration might be due to such a vascular change. Was degeneration being brought about through a reflex dilation of the vessels supplying the testis and if so, might not the same changes be produced through ligation of the internal spermatic vein by inducing passive congestion within the whole organ? Moore ('24*b*) also suggests that possibly some such relationship may occur. In an endeavor to demonstrate this relationship three pigs were operated; in two the internal spermatic veins were ligated and in the third the veins of the ductuli deferentes were ligated, care being taken not to occlude the ducts themselves. After seven days one of the pigs with internal spermatic vein ligature was killed. On the left side the ligature had not been made sufficiently tight to occlude the vessel, and the blood supply was found to be normal, but adhesions had followed handling the organ which was found within the abdominal cavity. In section this testis showed a very early stage in the degenerative process, earlier than that described for the six-day stage; and in this the contents were seriously deranged. Desquamation of the germinal epithelium had begun and spermatids were forming into giant cells in abundance. In some cases as high as forty nuclei could be seen together. On the right side, however, the testis was in the scrotum and the attached fat body was firmly adherent to the inguinal ring, thus preventing the return of the testis to the abdominal cavity. This testis was deeply cyanosed, the spermatic vein being completely occluded while the veins of the ductus deferens were greatly dilated. Microscopically this testis showed a vastly greater amount of hyperemia than any of the cryptorchid testes examined, yet there was nowhere the typical degeneration that was found in the abdominally-retained testes. There was no vacuolization nor emptying of the seminiferous tubules; instead, the tubular contents were considerably disarranged, swollen, and more or less coagulated in situ. The condition might well be compared to one of coagulation necrosis following infarction. The material stained very poorly with the stains which were being used. On the fourteenth day the other two pigs were

killed, and in the case of the second, operated as above, the left internal spermatic vein was also successfully occluded and here also a condition similar to the one just described was found. Many of the seminiferous tubules contained spermatozoa atypically arranged as were the other contents, with the whole staining poorly.

In the case where the vein of the ductuli deferentes were ligated a new venous return was well established along the vas, and the testes were in full spermatogenic activity.

These observations indicate that although the hyperemic state may be closely coupled with the process of degeneration it is certainly not the causative factor. It would be admissable to suppose that the hyperemia is the result of the degenerative changes, wherein the autolyzed products are more rapidly taken up by the blood stream. It was noticed repeatedly that leucocytes were abundantly distributed throughout the intertubular spaces, and in many instances they were present in large numbers in the tubules of the epididymis. They are here doubtless concerned with the phagocytic function, and the degenerating epithelium which has been liberated from the seminiferous tubules are in effect foreign proteins which have perhaps stimulated this phagocytosis.

E. Discussion.

This work was undertaken to determine if possible, just how the germinal epithelium which is desquamated from the seminiferous tubules in artifically cryptorchid testes is disposed of, and incidentally to ascertain something concerning the relative resistance of the various cells of the germinal epithelium to the degenerative process.

As to the effect of abdominal retention upon the sexually mature male testis I have but to confirm the findings of Moore ably described in recent articles. The organ is effected in such a way as to cause the cessation of all spermatogenic activity by the end of six days; at twenty days abdominal retention the seminiferous tubules have been almost completely denuded of their germinal epithelium, and succeeding changes relate chiefly to the size of the testis, the diameter of the tubules, and the

Sertoli cell content. It has been abundantly demonstrated (Moore, '22, 24*c*; Moore and Oslund, '24; Fukui, '23) that heat of certain temperatures will produce in the testis a degeneration identical with that seen in abdominally retained testes, and that the degeneration is identical in nature and proportional within certain limits to the degree of heat, whether it be applied by hot water, hot air, parafine, electric lights, etc. And further it has been shown (Moore and Quick, '24*a*) that the temperature within the abdomen is definitely higher than intrascrotal temperature, all of which logically lead to the conception of the scrotum as a necessary thermo-regulator for the testis as developed by Moore ('24*c*).

Bouin and Ancel ('03) believe they have demonstrated tubular degeneration attributable to vas ligation. Others (Moore and Quick, '24*c*; Oslund, '24, '25) have been unable to produce degeneration by this method when the testes were carefully kept within the scrotum. Although in my work, testes, the vas of which were ligated, were elevated to the abdominal cavity where degeneration took place as a result of subjection to an elevated temperature, the testes so ligated were in all respect like those retained in the abdominal cavity the vas of which were not ligated. It is not proposed to offer this evidence in confirmation or derogation of the results obtained by any of these workers, but simply as a statement that in conjunction with abdominal retention ligation is without apparent effect.

It was pointed out in the text of this paper that although many of the epithelial cells in the seminiferous tubules are partially or almost completely autolyzed in situ, many others are thrown into the lumen of the tubule almost unchanged. These cells were followed through the straight tubules and rete to the proximal tubules of the epididymis where they are seen intermixed with spermatozoa already present previous to the elevation of the organ of the abdominal cavity. Obviously these invading cells, together with the spermatozoa already present must become absorbed in the epididymis, remain there, or be evacuated through the genital passages. Were the latter to take place the spermatozoa would be pushed on ahead probably mixed with some of the degenerating cells, followed by the

newly acquired degenerating epithelium which should be found all along the passage. Were the whole to remain unchanged the epididymis would be ballooned up with both the old and newly acquired contents. The fact is the most of the spermatozoa present in the epididymis at the time of operation remain there, and are seen in various stages of degeneration within this organ. Likewise other germinal cells may be identified in the epididymis undergoing a more rapid degeneration than that seen in the case of the spermatozoa. We are left with little doubt that practically all the germinal epithelium of the testis and spermatozoa in the epididymis are absorbed directly from these organs into the blood stream after degeneration and liquefaction. As a further proof of this, if any is needed, spermatozoa which escape into the vas deferens which is markedly less vascular than the epididymis, require a much longer time for absorption than those retained in the ductuli efferentes.

And what of the effect of all this absorption on the organism? McCartney ('23) states that he was able to produce sterility in rats through injection of spermatozoa, and in a second article he states: "In the male rat vasoligation may be followed by the development of antibodies for semen in the blood." It is admitted that if the first is true the second would seem plausible. In my work ten pigs were operated in which one testis was elevated to the abdominal cavity, the opposite retaining its normal relation in the scrotum. The operated testes were allowed to remain for from six to sixty-four days in the abdominal cavity. Varying amounts of degeneration had taken place with the absorption of these products together with the spermatozoa present at the time of operation, and in not a single case was the spermatogenic activity in the opposite testis affected, nor was there any noticeable change in the testicular or epididymal contents from the normal.

Concerning the viability of spermatozoa so much has been said that any attempt to review the literature here would be futile. Be it said that reports vary from a few hours for the boar sperm in the sows uterus (Anderson, '22) to 21 days for the human sperm in the female uterus (DeLee, '21). In my observations I have found a fairly definite relation between

viability and a tendency toward clumping. Spermatozoa were found alive in the ductus epididymis, and the vas for nine days after elevation of the entire testis to the abdominal cavity, and since it is reasonably certain that spermatogenic activity ceases on elevation of the organ, and since the spermatozoa pent up in the vas and epididymis were in these locations some time previous to operation, it follows that the length of life of a fully mature spermatozoön is something more than nine days. Just how long a spermatozoön remains in the seminiferous tubule after maturity, and what time is required for its passage into the vas is a question which must be answered before we can state anything more definite concerning the length of life of fully developed sperm.

As to the rôle played by the vascular changes in the degenerative process it must be admitted that insufficient work has been done in this direction to permit of any definite statements. However, from the meager data here presented it would appear that hæmostasis induced by ligation of the blood supply to the testis produces a coagulation necrosis of the germinal epithelium, not followed by a removal of the germinal epithelium at least within sixteen days. Hence we are lead to believe that the vascular changes seen in artifically cryptorchid testes is a case of active hyperemia, and that through this agency the degenerating elements are rapidly taken into the blood stream.

F. Summary and Conclusions.

1. When the testes of sexually mature male guinea pigs are elevated to and allowed to remain in the abdominal cavity, a progressive desquamation and degeneration of the germinal epithelium takes place; at six days abdominal retention degeneration is well marked, at ten days well advanced, and at twenty days practically all epithelium has been removed. This degeneration can be directly attributed to the increased temperature of the abdominal cavity over that of the scrotum.

2. Neither the speed nor the nature of the degenerative process is effected by ligation of the ductus deferens of the cryptorchid testis.

3. All of the degenerating elements from the seminiferous

tubules are absorbed in the testis proper, or are carried to the epididymis where they are absorbed along with the greater part of the spermatozoa which were present at the time the testis was elevated to the abdominal cavity.

4. Absorption of the germinal epithelium from a cryptorchid testis into the blood stream does not affect the integrity of the scrotally-retained normal testis.

5. Spermatozoa will remain alive in the germinal passages of the guinea pig for longer than nine days. Following their loss of motility they rapidly lose their tendency to clump, but degeneration of mature spermatozoa takes place slowly, and especially so in the ductus deferens. It has been determined that a given spermatozoön in the male genital passages though retaining its capacity for motility for nine days, may retain its morphological identity for over 100 days.

6. Venous stasis through ligature of the internal spermatic vein of testes allowed to remain in the scrotum does not produce the degeneration complex seen in testes retained in the abdominal cavity.

LITERATURE CITED.

Anderson, W. S.

'22 Vitality of Spermatozoa. Ky. Agr. Exp. Sta. Bul. 239.

Armstead, Reo B.

'25 Structure Function and Regeneration of Seminal Vesicles of Guinea Pigs. Jour. Exp. Zoöl., Vol. 41, p. 215.

De Lee, Jos. B.

'21 Prin. and Practice of Obstetrics, p. 21.

Fisher, N. F.

'23 The Influence of the Gonad Hormones on the Seminal Vesicles. Am. Jour. Phys., Vol. 64, p. 244.

Fukui, N.

'23 On a Hitherto Unknown Action of Heat Ray on the Testicle. Japan Med. World, Vol. 3, No. 2.

'23 Action of Body Temperature on the Testicle. Ibid., Vol. 3, No. 7.

'23 On the Action of Heat Rays upon the Testicle: An Histological Hygienic and Endochrinological Study. Acta Scholæ Medicinalis Universitatis Imperialis in Kioto, Vol. 6, Fasc. 11, p. 225–228.

Hoehne and Behne.

'14 Uber die Lebensdauer homologer und heterologer Spermatozoen im weiblichen Genitalapparat. Zentralblatt fur Gyn. (Quoted from Williams Obstetrics. 1923.)

Lewis, L. L.

'11 The Vitality of Reproductive Cells. Bul. 96, 1911. Oklahoma Agri. and Mech. College.

McCartney, J. L.

'23 Studies on the Mechanism of Sterilization of the Female by Spermatoxins. Amer. Jour. Phys., Vol. 63, p. 207.

'23 Further Observations on the Antigenic Effect of Semen. Ibid., Vol. 66, p. 404.

Moore, Carl R.

'22 Cryptorchidism Experimentally Produced. Proc. Soc. Zoöl. Anat. Rec., Vol. 24, p. 383.

'24*a* The Behavior of the Testis of Transplantation, Experimental Cryptorchidism, Vasectomy, Scrotal Insulation, and Heat Application Endocrinology, Vol. 8, No. 4.

'24*b* Properties of the Gonads as Controllers of Somatic and Psychical Characters. VI. Testicular Reactions in Experimental Cryptorchidism. Am. Jour. Anat., Vol. 34, No. 2.

'24*c* Properties of the Gonads as Controllers of Somatic and Psychical Characteristics, VIII. Heat Application and Testicular Degeneration, the Function of the Scrotum. Am. Jour. Anat., Vol. 34, No. 2.

Moore, Carl R., and Oslund Robt.

'24 Experiments on the Sheep Testis. Cryptorchidism, Vasectomy and Scrotal Insulation. Am. Jour. Phys., Vol. 67, pp. 595–607.

Moore, Carl R., and Quick, Wm. J.

'24*a* The Scrotum as a Temperature Regulator for the Testis. Am. Jour. Phys., Vol. 68, pp. 70–79.

'24*b* The Properties of Gonads as Controllers of Somatic and Psychical Characteristics. VII. Vasectomy in the Rabbit. Am. Jour. Anat., Vol. 34, No. 2.

Nürnburger.

'20 Klin und exp. Untersuchungen uber die Lebensdaur der menschlichen Spermatozoen. Monatschr. f. Geb. und Gyn. Quoted from Williams Obstetrics, 1923.

Oslund, Robt. M.

'24 A Study of Vasectomy of Rats and Guinea Pigs. Am. Jour. Phys., Vol. 67, pp. 422–443.

'25 Vasectomy on Dogs. Am. Jour. Phys., Vol. 70, p. 117.

Williams.

'23 Obstetrics. P. 96. D. Appleton & Company.

Wolf.

'21 The Survival of Motility in Mammalian Spermatozoa. Am. Jour. Agri. Science, Vol. 11, 1921. Quoted from Marshalls Physiology of Reproduction, 1922.